nature

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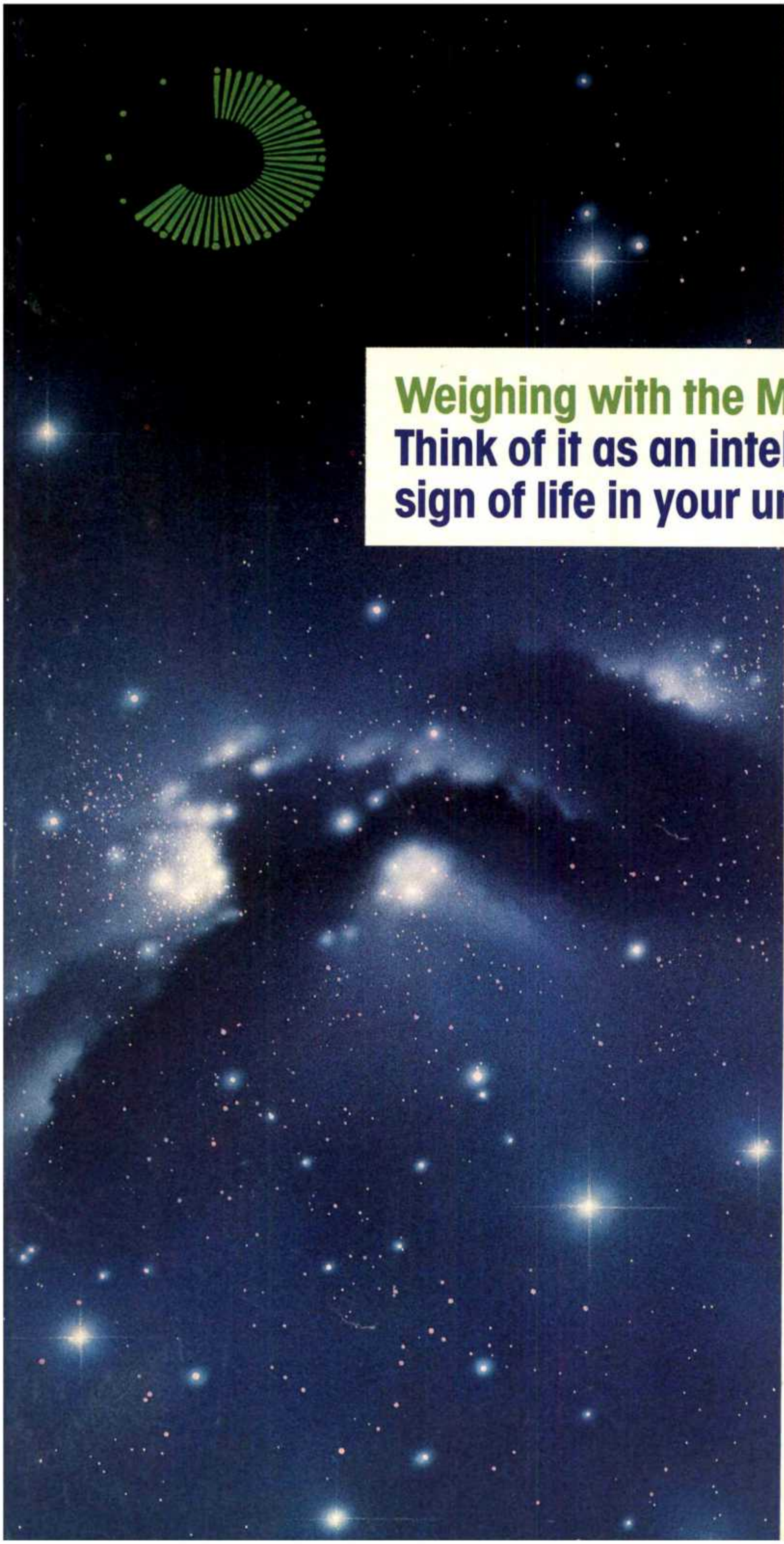
A second class of tRNA charging enzyme

Predicting the structures of solids

INSTRUMENTATION product review



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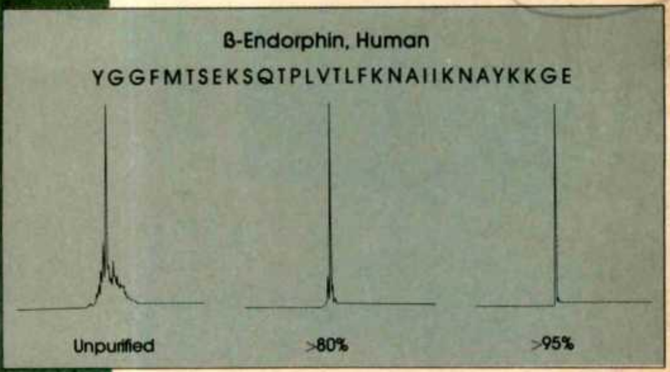

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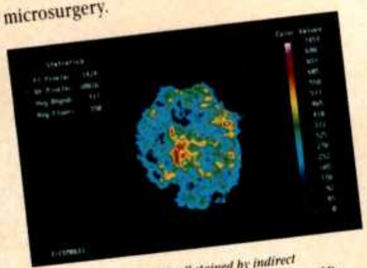

HIV-1RF infected CEM-SS cell stained by indirect immunofluorescence using anti-gp 120. (Courtesy of Dr. Jonathan T. Warren, National Institutes of Health)

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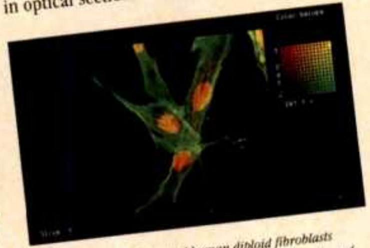

Optical slice (.5μ) of normal human diploid fibroblasts stained with NBD phallacidin showing actin filaments, and propidium iodide showing nuclear areas, using the ACAS confocal accessory. (Courtesy of Asmina H. Jiwa, Meridian Instruments, Inc.)

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Transient increase in intracellular Ca^{2+} concentration induced by potassium depolarization in a hamster insulin-secreting tumor cell. (Courtesy of Dr. H. A. Pershadsingh, Lab for Cell Analysis, University of California, San Francisco, and Dr. Margaret H. Wade, Meridian Instruments, Inc.)

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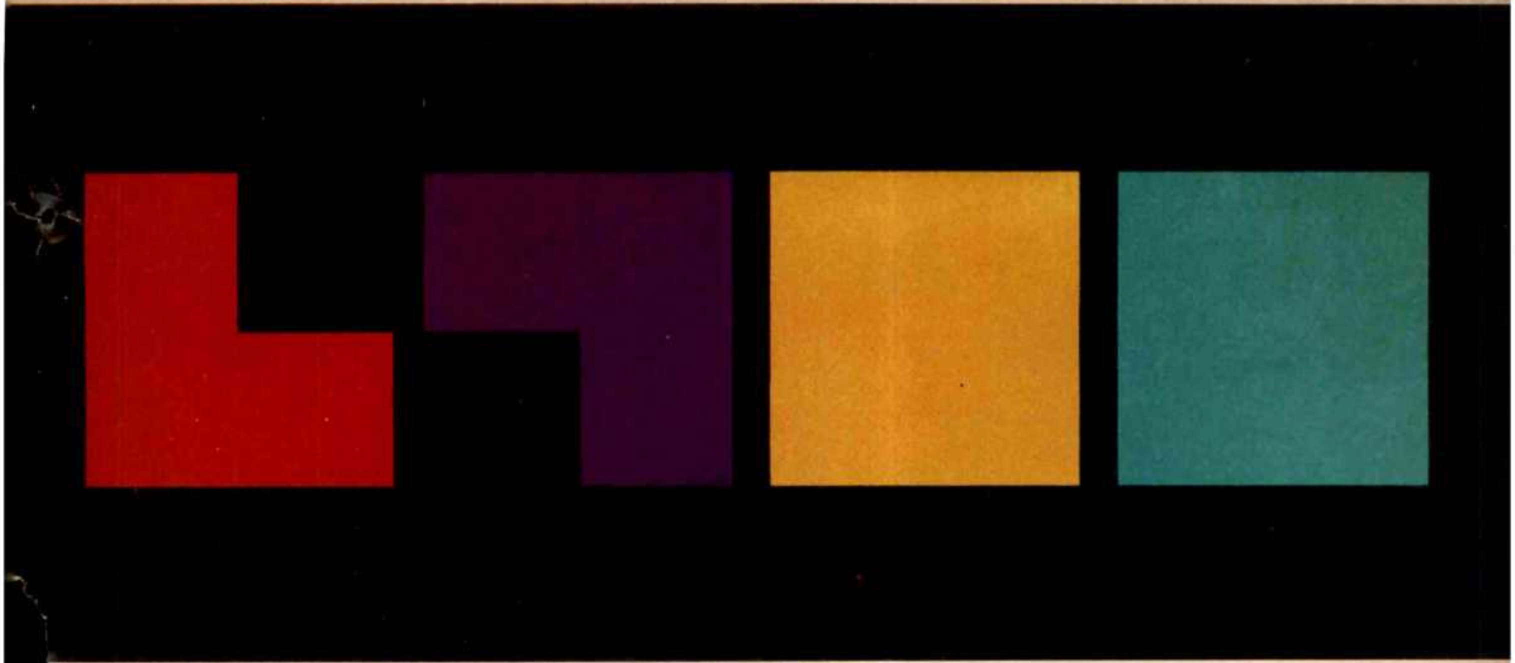

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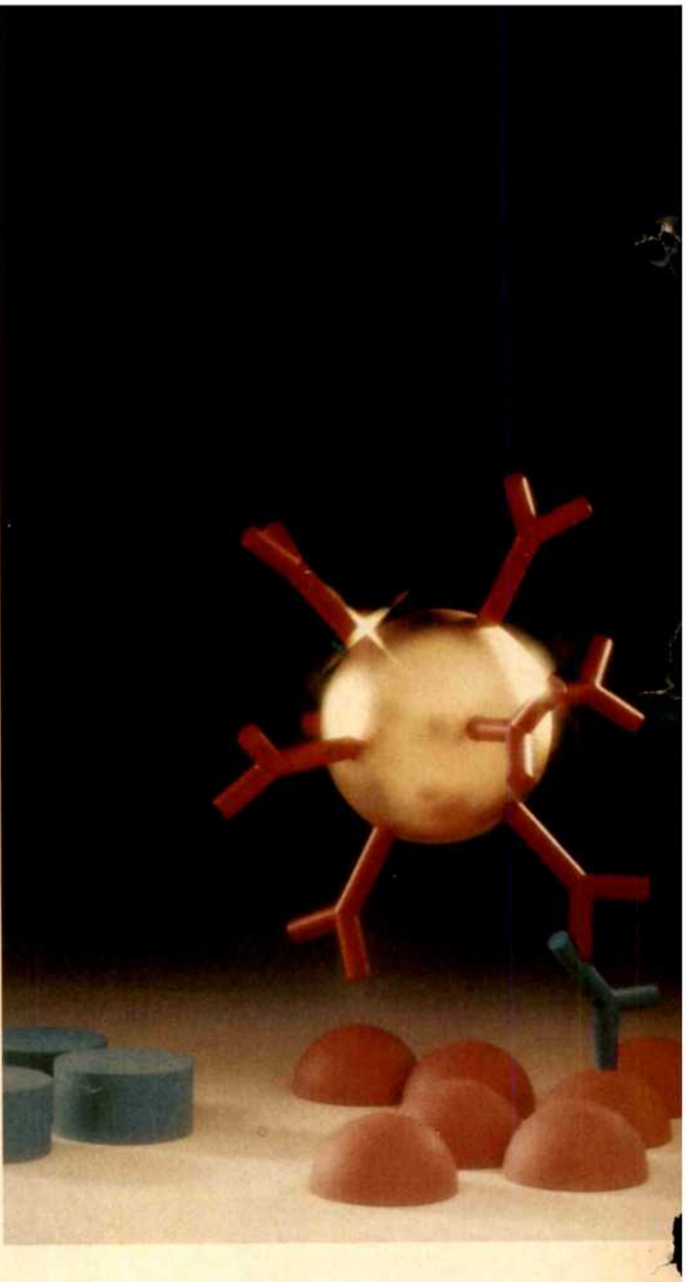

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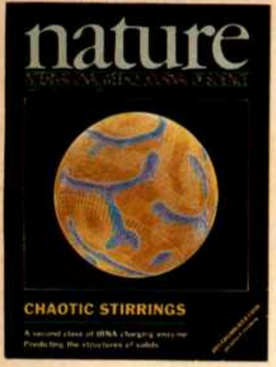

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20 September 1990
Vol. 347 Issue no. 6290

◄ An illustration of horizontal velocities (arrows) and temperatures (colours) in a computer model of convection in the Earth's mantle. Although the model does not include lithospheric 'plates', it exhibits planar downwellings reminiscent of subduction zones on the Earth which evolve chaotically with time. Similar behaviour of descending slabs at subduction zones may influence the dispersal and aggregation of continents. See page 274.

THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

Evolutionary surprise

The crystal structure of seryl-tRNA synthetase is found to be highly unusual and quite unlike the known structures of enzymes that charge tRNAs with amino-acids. The implication is that different subsets of tRNA-charging enzymes may have separate origins. Pages 249 and 231.

Crystal ball

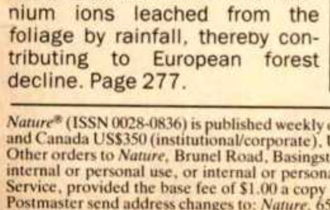
The rapid expansion in computer power in recent years has made possible the accurate prediction of crystal structures of inorganic solids. Zeolites, important as catalysts in the petrochemical industry, and semiconductors are now within the capabilities of the techniques. Page 243.

Holed gold

Implantation of gold with helium ions produces an ordered 'superlattice' of helium bubbles 2 nm in diameter and a 'macrolattice' of 60-nm bubbles, both ordered with the same symmetry as the gold lattice. Ion implantation should provide insight into the erosion resistance of materials for use inside future nuclear fusion reactors. Page 265.

Needle match

Co-deposition of ammonia and sulphur dioxide in conifers exposed to atmospheric sulphur dioxide may increase nitrogen concentrations in pine needles and the concentration of ammonium ions leached from the foliage by rainfall, thereby contributing to European forest decline. Page 277.



Droplets of moisture forming on a spruce shoot — site of the acid deposition.

Living microscopy

The structure of single living cells and chromosomes can now be studied with a higher resolution than previously possible in a technique that combines Raman spectroscopy with confocal microscopy. Pages 301 and 230.

Melting pointless?

Recent observations have revealed a high electrical conductivity in the asthenosphere, prompting speculation that partial melting of olivine in that region of the upper mantle might be the cause. But the presence of a small amount of hydrogen might have a similar effect. Pages 272 and 232.

Conservative policy

Cloning of the murine homologue of the human neurofibromatosis (NF-1) gene reveals a striking conservation throughout evolution, and significant homology with yeast IRA inhibitory regulator proteins of the ras–cAMP pathway. The possible role of NF-1 as a negative regulator of mammalian signal transduction increases speculation that it is a tumour suppressor gene. Page 291.

Broadening spectrum

A new family of GTPases described on page 256 is involved in a wide range of physiological processes requiring microtubule movement or protein sorting. The brain protein dynamin, a microtubule-activated nucleotide phosphatase, shows similarities with a family of antiviral factors induced by interferon, and a vacuolar protein-sorting factor from yeast. News and Views, page 229.

Misspent youth

In the collared flycatcher, *Ficedula albicollis*, costly reproduction early in life accelerates the decline in fertility with increasing age, as is predicted by at least one theory for the evolution of senescence. Page 279.

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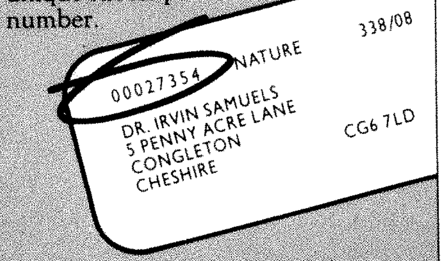

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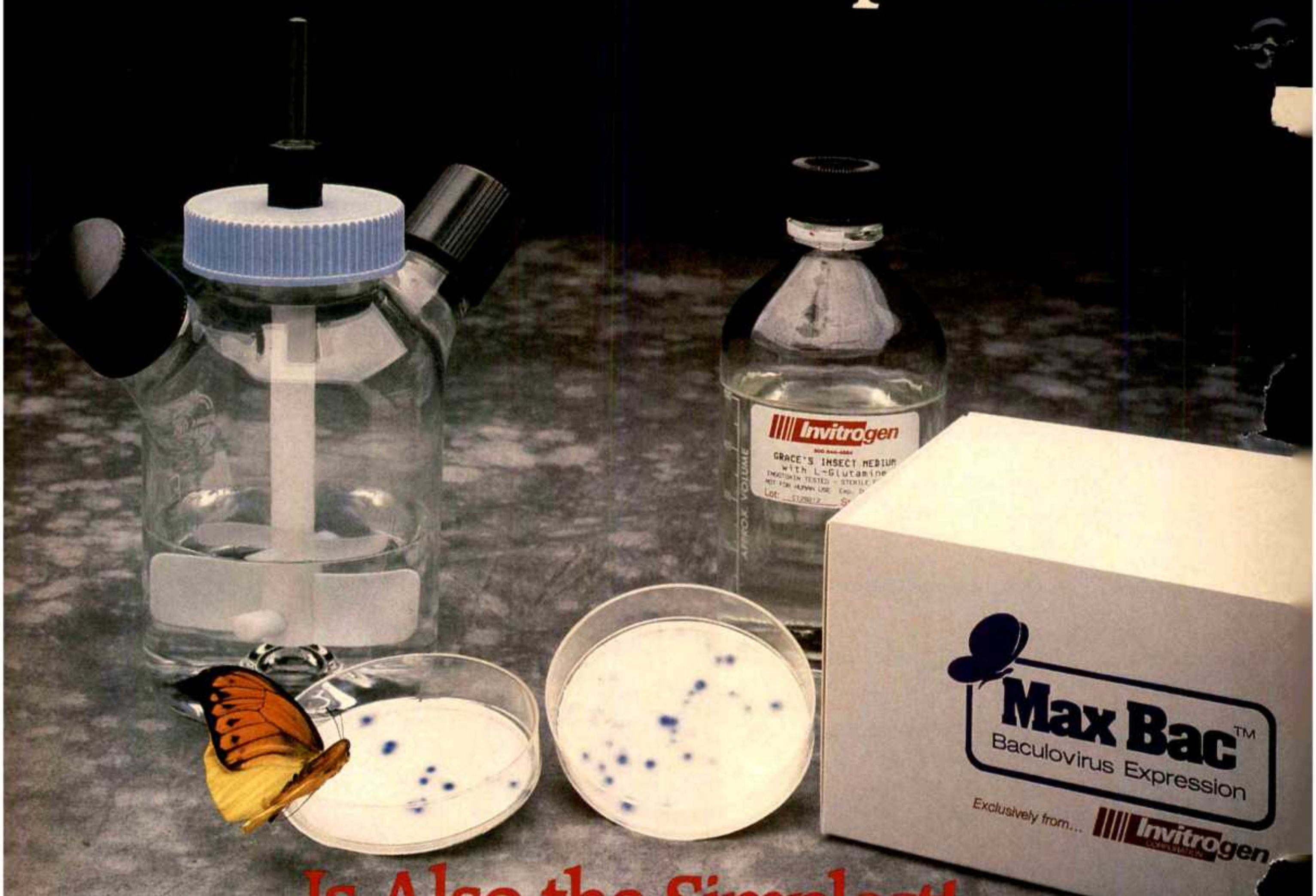

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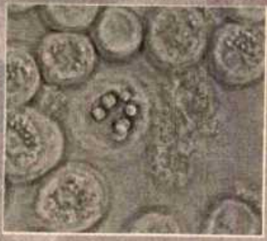

Figure 1. *Infected Sf9 insect cells showing viral occlusions.*

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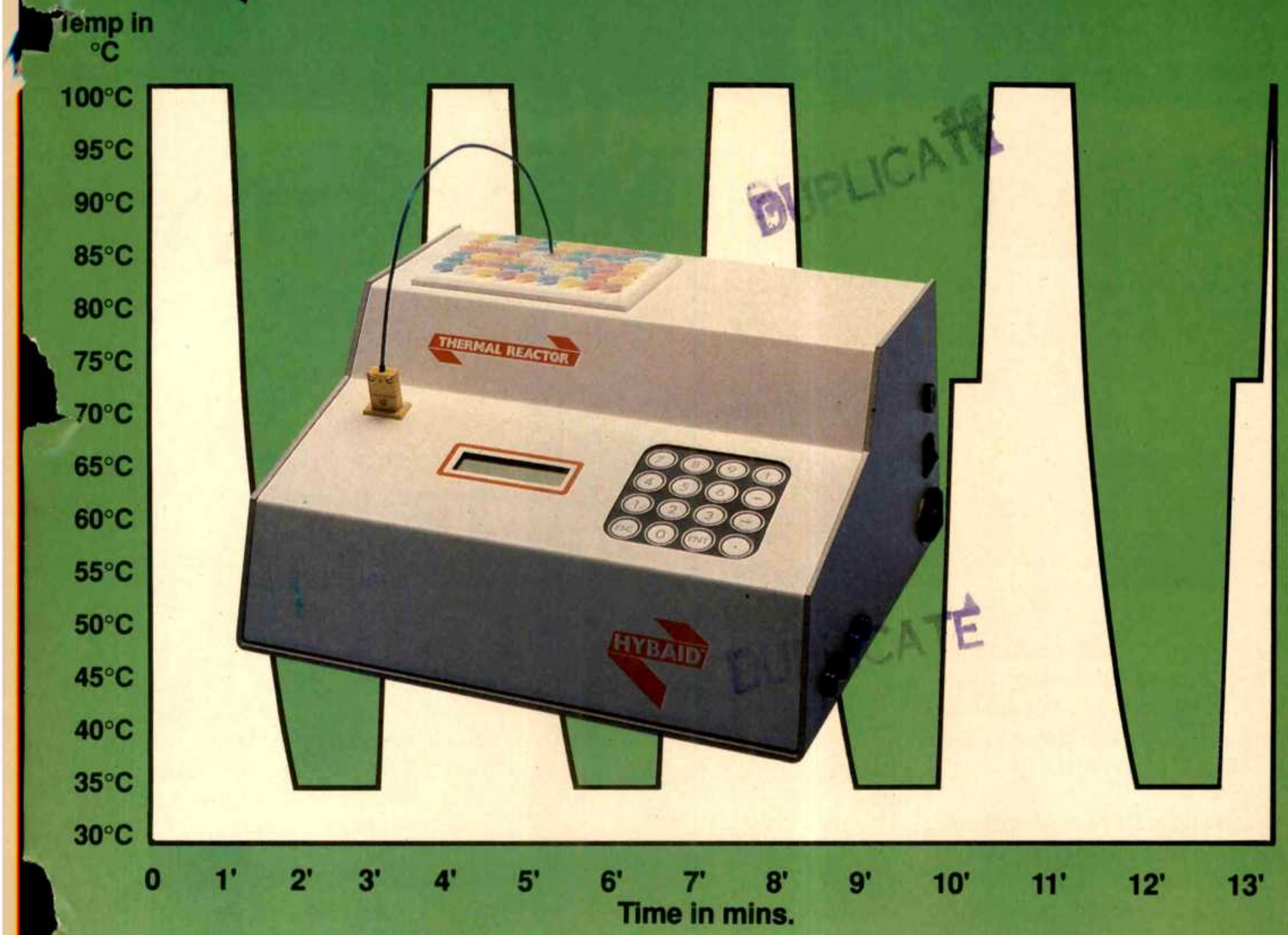

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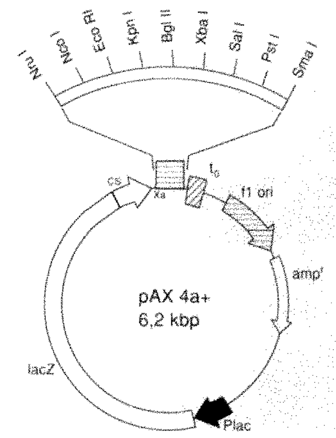
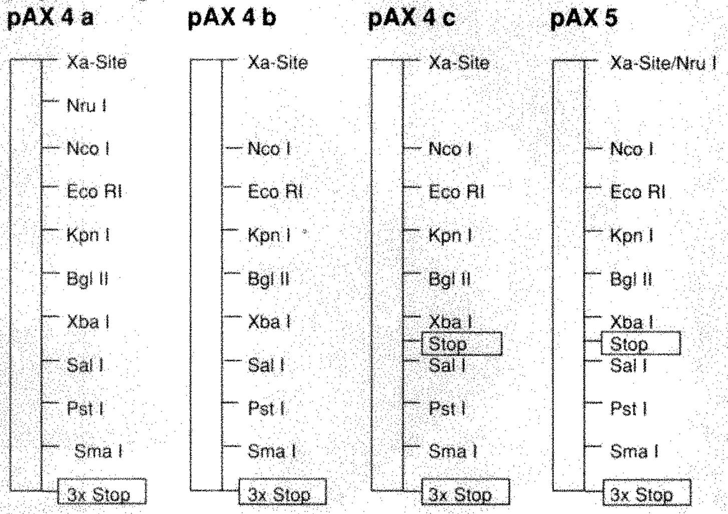


Xa: Xa cleavage site
Cs: collagen fragment
t_0: lambda to terminator
f1: phage f1 intergenic region

polylinker regions of:



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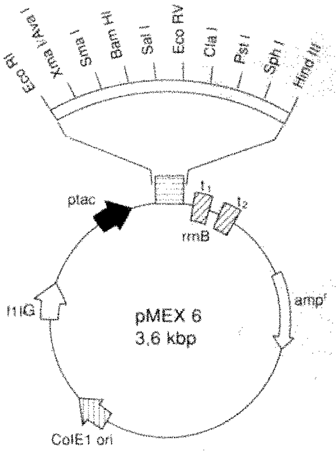

t_1, t_2: terminator of the rrnB operon
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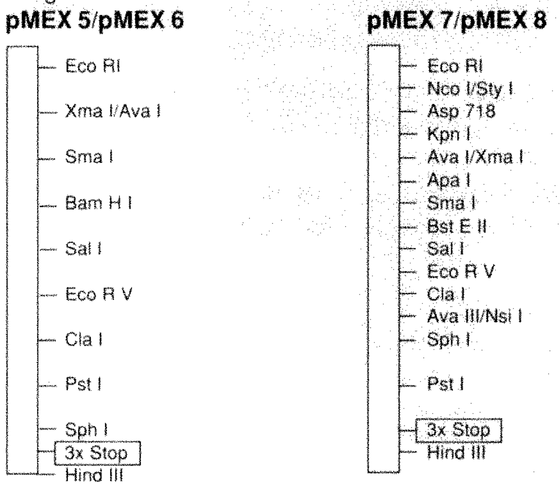

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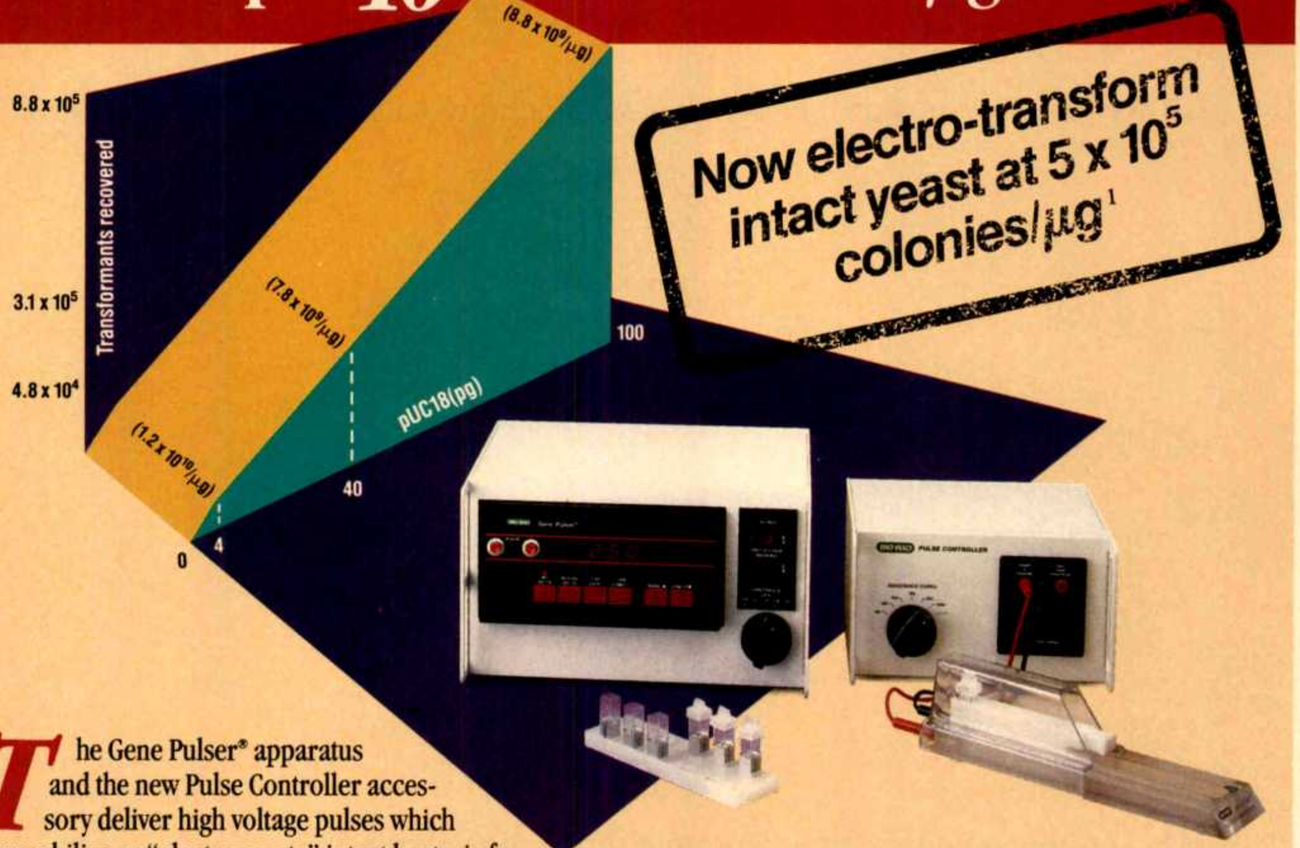

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*LE392 transformed with pUC18

References

1. Becker, D.M. and L. Guarente, *Meth. Enzymol.*, in press.
2. Dower, W., J. Miller and C. Ragsdale, *Nucl. Acids Res.*, 16, 6127 (1988).

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Answers.

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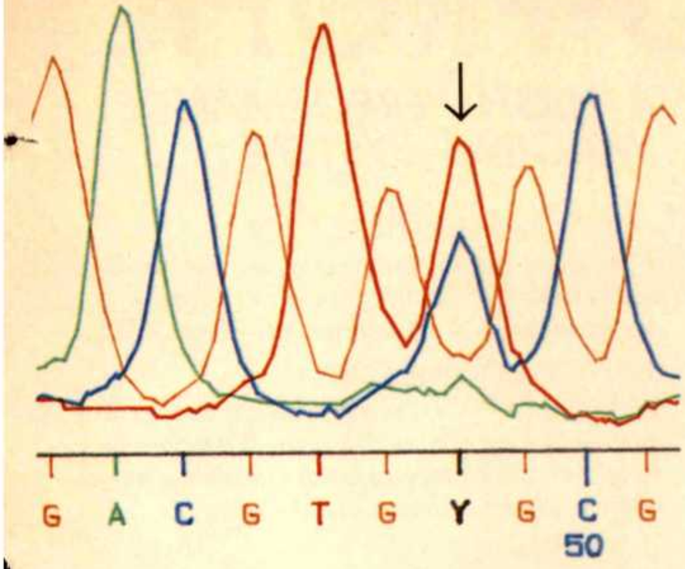
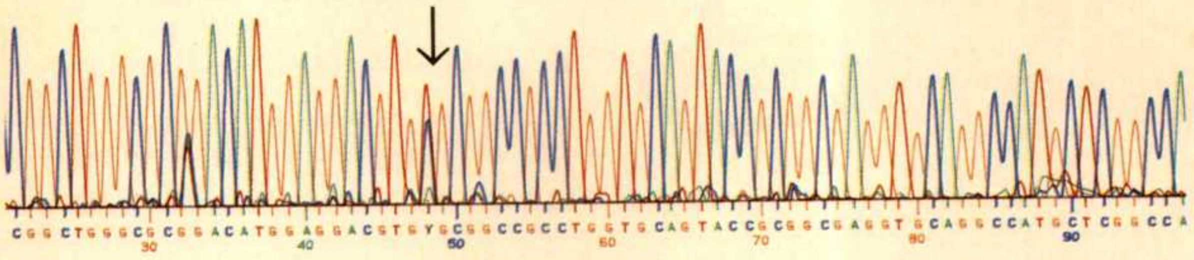


Direct genomic solid phase sequencing of the human apolipoprotein E gene for diagnosis of a heterozygotic point mutation, Hultman and Uhlén (1990) submitted for publication.

Raw DNA sequencing data



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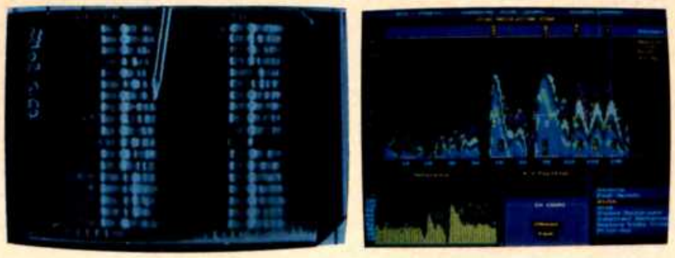

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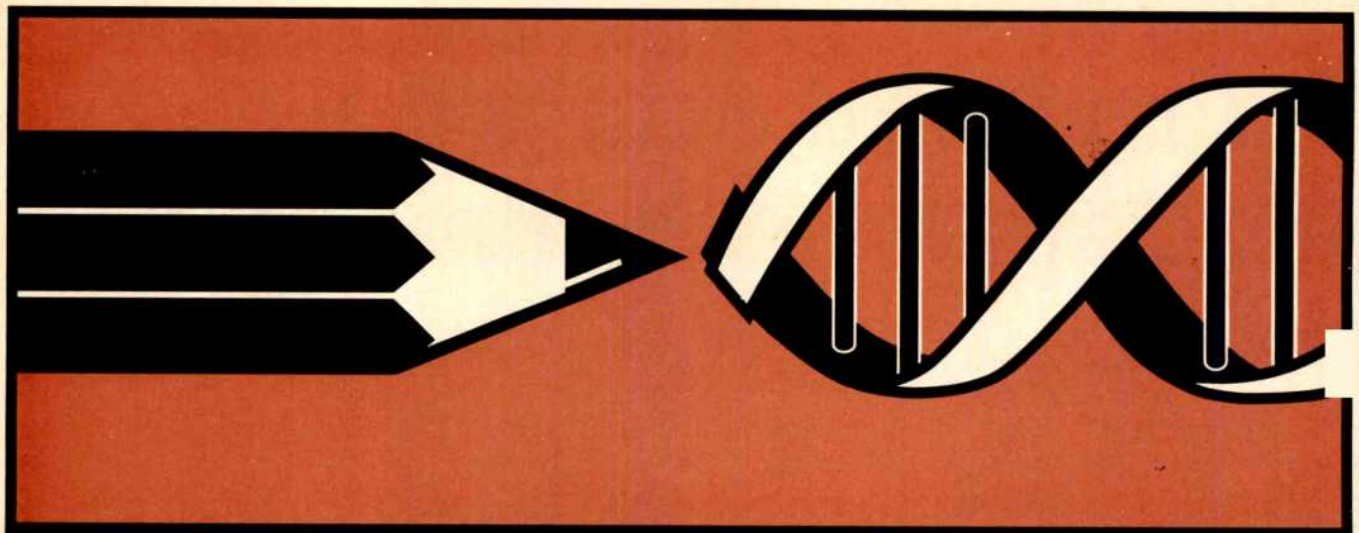

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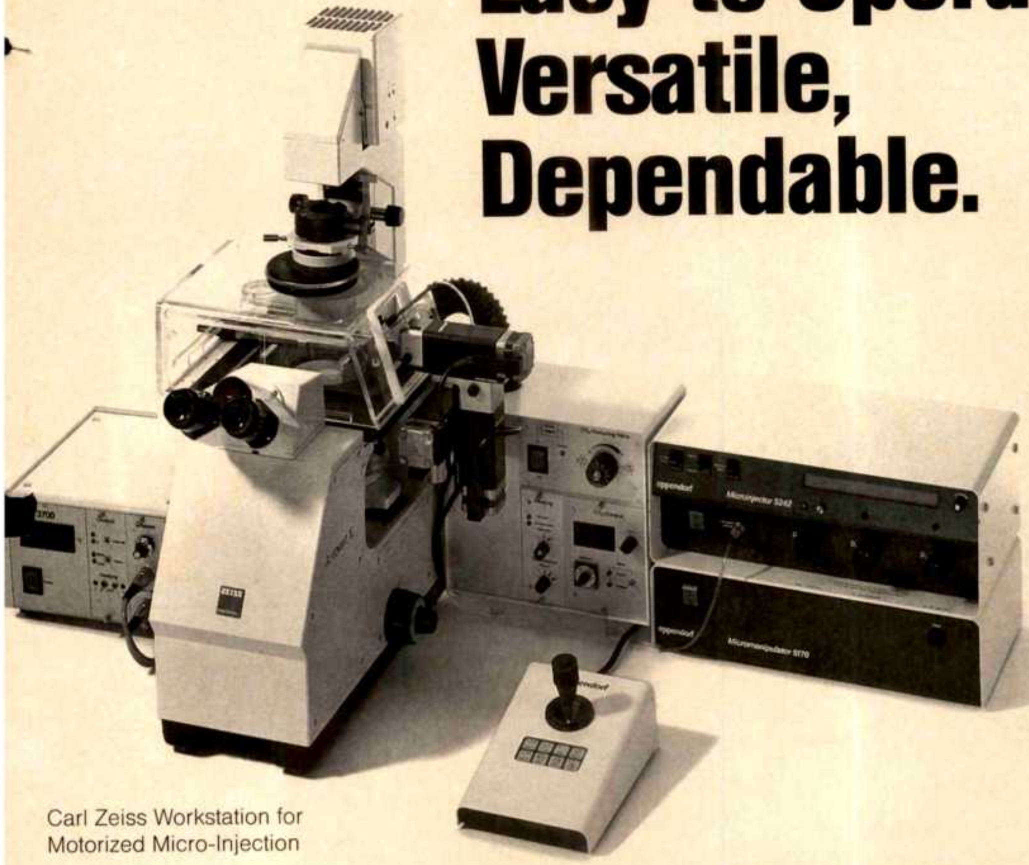

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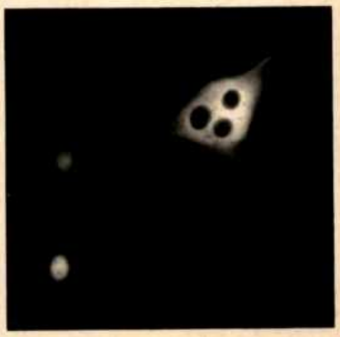

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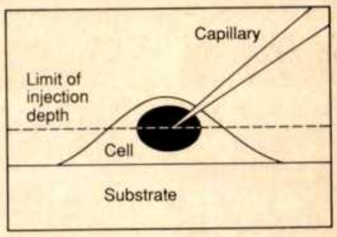

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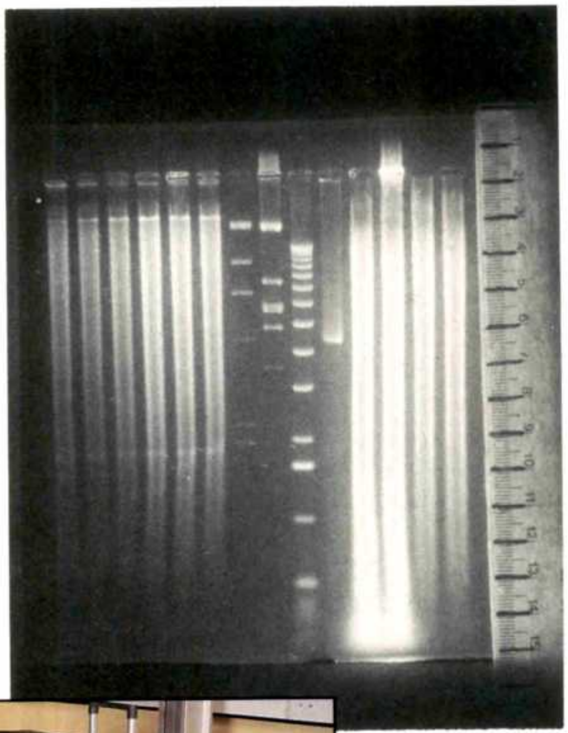
Polaroid at work Nº 25

DNA analysis and instant photography

AT ST GEORGES Hospital Medical School, Tooting, research into hypertension and diagnosis of genetic diseases, such as cystic fibrosis, is based on an analysis of DNA sequences - and instant photography techniques are assisting scientists in these areas of work.

Chief technician Jackie Barley of the Child Health Department is involved in research to find out whether allelic polymorphisms of candidate genes can be associated with essential hypertension. Research and medical evidence suggests that up to 60 per cent of hypertension is attributed to genetic influence. Genes with known products that effect blood pressure, renin and atrial natiuretic factor are being studied for association with hypertension. The study includes hypertensive patients having a family history of hypertension with control subjects and subjects representing the upper and lower quintiles for diastolic pressure from randomly drawn samples of Afro-Caribbean and Caucasian subjects.

Part of the research is to identify and size polymorphic DNA sequences. This is assisted by Polaroid's MP-4 camera which is used to photograph digested DNA fragments separated by electrophoresis in agarose gels. These are stained with ethidium bromide and illuminated with UV light.



Right: Digested DNA fragments, photographed on Polaroid Type 667 instant print film, using the MP-4.

Below: Jackie Barley, working with the MP-4 at St. Georges Hospital Medical School.

Barley needs Polaroid photography to give a permanent record of this part of the research. She explained: 'One of the reasons for using Polaroid photography is that you can check the quality of the restriction enzyme digest. Incomplete digestion and overloading of the sample will result in extra false bands on the autoradiograph.

'Known DNA size markers are also run on the gels and when photographed alongside a ruler provide an instant permanent record allowing DNA fragment sizes to be determined later.'

The separated DNA fragments are transferred from the electrophoresis gel and immobilised onto a nylon membrane using conventional blotting techniques (ref: E.M. Southern, *Journal of Molecular Biology* 1975, Vol 98, pages 503-517). Radioactive c-DNA gene probes are hybridised to the complimentary sequences of DNA on the membrane and visual-

'It's reassuring to have a permanent visual record'

- Jackie Barley, St Georges Hospital Medical School.

CONTINUED OVER THE PAGE ►

The multi-purpose MP-4 copy camera

Above: The Polaroid MP-4 camera system and film.

POLAROID's MP-4 multi-purpose camera is a complete photographic studio in miniature, a camera and copy stand combined for making instant images on more than two dozen instant films, including 35mm, $3^1/_4$ x $4^1/_4$-inch, 5 x 4-inch and even 10 x 8-inch formats when upgraded.

The unit can be adapted to handle a wide range of applications, including specimen photography, photomicrography, photomacrography and general copy work. The camera body slides on a column over a baseboard, or it can be used, independent of its copying stand, as a conventional studio camera.

Features include eye-level reflex viewing, six interchangeable flat field lenses, self-cocking shutter, easily interchangeable film holders, infinitely-adjustable built-in lighting and a camera head that rotates through 360 degrees.

The modular design of the camera, together with its wide range of accessories, means that photographers can build a system tailor-made to specific requirements, with the option of upgrading as needs change.

Peel-apart print films

THE MP-4 takes any one of Polaroid's vast range of $3^1/_4$ x $4^1/_4$-inch pack films. According to the film in use, they offer instant images in black and white or colour.

Processing is by Polaroid's peel-apart method. Two tabs are pulled from the film back, as soon as the exposure has been made and the print emerges with the second one.

After a short wait, the print is peeled away from its backing. Development time, on average, is 30 seconds for black and white, 60 seconds for colour.

There are over fifty different films in Polaroid's range. Type 669 is just one, available in glossy or the Type 669S silk finish surface. The silk finish is one that has, since its launch, proved tremendously popular with many different types of photographer.

It is less reflective, less prone to marring by finger-prints and dries faster than its glossy counterpart, so that it can be mounted in a presentation folder or safely stacked with other prints immediately after exposure without the risk of sticking.

DNA ANALYSIS AT MEDICAL SCHOOL

◀CONTINUED FROM PREVIOUS PAGE

ised by exposing it to x-ray film to create an autoradiograph.

As Jackie Barley says: 'Polaroid photography is actually essential. There are so many procedures that can go wrong, it's reassuring to have a permanent visual record to refer to.

The Polaroid camera is also used by senior molecular geneticist Rohan Taylor in prenatal genetic disease diagnosis. A key area of this work is cystic fibrosis analysis, where DNA is analysed from chorionic villus (placenta) samples.

Rohan Taylor uses the Polaroid MP-4 to photograph agarose gels with amplified DNA fragments stained with ethidium bromide. These gels have a limited fluorescent time before the bands irrecoverably fade. Again, the photographs highlight band locations, showing differences in DNA sequence and indicating the presence of mutations in the cystic fibrosis gene. She also uses the camera for muscular dystrophy diagnosis (where the absence of an amplified DNA fragment is indicative of the disease state).

The system is now the recognised method for documentation and publication of electrophoresis gel analysis.

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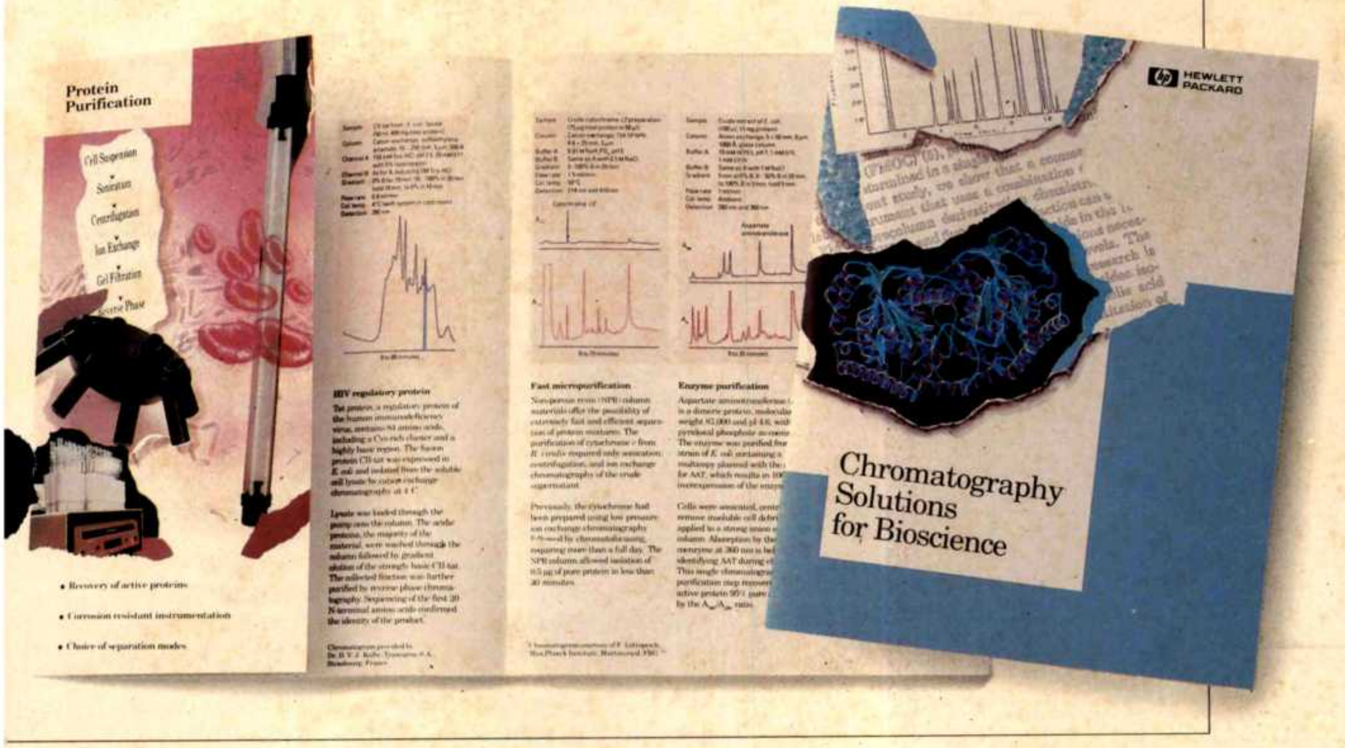

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GENERAL

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Acknowledgements should be brief. Grant numbers and contribution numbers are not allowed.

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NPT in serious trouble

Once again, a review conference of the Nuclear Non-Proliferation Treaty has ended in disarray. But this time, the consequences could be serious.

JUST before dawn last Saturday, the quinquennial review conference of the Non-Proliferation Treaty (NPT) at Geneva reached the dispiriting conclusion that it could not agree on a final statement embodying the conclusions reached in the preceding two weeks. While the immediate cause of failure was the insistence of the Mexican delegation that the signatory nuclear powers should commit themselves to a comprehensive nuclear test-ban treaty by the time of the next review conference in five years, the underlying trouble seems to have been the general ill-temper of the non-nuclear signatories.

Yet is this not the year in which the nuclear signatories will be able to make good their promise to "achieve at the earliest possible date" an end to the "nuclear arms race", "nuclear disarmament" and "general and complete disarmament under strict and effective international control"? Since the 1985 review conference, after all, the Soviet Union and the United States have signed and ratified the treaty on nuclear missiles of intermediate range (INF). Will there not now also be the START treaty to limit Soviet and US strategic weapons, as well as an agreement on conventional forces in Europe?

That was the outlook when this year began, as it was at the superpower summit in Malta earlier in the year. But the prospects have faded as the months have passed. Europeans may have been cheered by the good things that have happened in the past few months in Central Europe, but there is no reason why NPT signatories elsewhere should be buoyed up by that good fortune. Instead, they have the trouble in the Gulf to worry them.

The Gulf, or the Middle East in general, is not irrelevant to the NPT. Israel (not a signatory of the treaty) is assumed to be a nuclear power on the strength of its technical capability, its secretiveness about its activities at the Damona site in the Negev and the calculation (in which there is some force) that nuclear weapons might effectively be used, in the last resort, to give pause to a seemingly successful conventional attack. The arguments used since 1945 by acknowledged nuclear powers could well have persuaded other Middle East states to follow suit. So, naturally, there has from time to time been talk of building an "Islamic bomb", usually centred on the alleged ambitions of Pakistan (which, like India, is not a signatory of the treaty). If more is not done, there will be one some day.

Yet Iraq is dutifully a signatory, as are Israel's other neighbours such as Egypt, Jordan and Syria. So (further away) are Iran and Kuwait. The only notable exception is Saudi Arabia. Yet Iraq was accused by Israel in 1981 (when it bombed out of action the research reactor supplied by France) of nursing nuclear ambitions. Circumstantial evidence since then, as well as the evident fascination of the Iraqi leadership with elaborate and unconventional weapons systems (not to mention poison gas), suggests that the accusation may have been correct.

That does not, of course, excuse the illegal bombing of the reactor; it would have been more effective if evidence of Iraq's intentions had been used to ensure that the International Atomic Energy Agency (IAEA) carried out a "special" inspection as allowed under the terms of its agreements with signatory states. That might even have forestalled some of the present trouble.

For the long haul, and for the long-term future of the NPT, persisting uncertainties in the Middle East are a literally frightening example to other regions of the world. Whether Israel and one or more of its potential adversaries are nuclear powers may be less important than the perceptions of states elsewhere. Some of them may reflect that if it is now the case that, for a nation to walk tall in a violent world, its adversaries must be made to regard it as putatively nuclear, they had better take appropriate steps themselves. In short, the example of the Middle East and, more generally, the likelihood that the waning of superpower equipartition of influence will allow regional conflicts to become more common, has eroded previous restraints on military nuclear development. The pressure in that direction is increased by the continuing self-exclusion from the NPT of China and France, both acknowledged nuclear powers, and by the likelihood that the years ahead will put a premium on civil nuclear power generation.

In the circumstances, Mexico's protest at Geneva was not merely unrealistic but counter-productive. If Mexico's objective was to strengthen the non-proliferation regime, it chose an odd way to that end. To demand that governments should agree to agree, and on the spot, is to deny the realities of international relations, however much they have improved. Only a little reflection would have shown that a comprehensive test-ban would be a curious animal without the adherence of France and China, which the signatories of the NPT are not in a position to command.

Moreover, the experience of the past few years has shown that major arms control agreements depend on the undistracted eagerness of powerful leaders for a deal. Without the enthusiasm of Mr Mikhail Gorbachev, there would not have been an INF treaty. He may yet be able to pull a START treaty and a conventional weapons agreement out of the hat, despite the obstacles erected by the Soviet military (over submarine-launched cruise missiles and aircraft respectively), but the signs are not as good as they were at the beginning of the year. For Mexico to have scorned the compromise offered at Geneva last week, which would have committed all the signatories to some kind of activity, was principled to the point of perversity.

So what is to be done? The next conference is that at which the NPT will lapse unless its signatories agree to renew it. That would be a tragic outcome, especially because of the constructive proposals for operating and interpreting the NPT agreed last week. These include a proposed undertaking that nuclear suppliers should provide nuclear materials only to countries accepting international safeguards on all their nuclear plants and even a "negative assurance" that nuclear states will not use nuclear weapons against non-nuclear countries.

Luckily, the treaty as it stands allows a procedure for amendments to be proposed by any signatory and one by which a third of the signatories can require an *ad hoc* conference to consider them. The obvious next step is that some well-intentioned government should offer as an amendment the conference statement as it might have been. When tempers have cooled a little, even Mexico might consider that a valuable way of making amends. Otherwise, some other state should take the initiative. To let the fate of nuclear proliferation rest on governments' separate interpretation of last week's aborted review conference is too dangerous. Then, Mexico will have made a debating point and ruined the most durable of all the arms control agreements. □

Bad luck insurance

Confidentiality will complicate attempts to sequence the human genome in ways that should be faced.

REPRESENTATIVE John Conyers (see page 221) is not alone in worrying about the use that will be made of information relating to the nucleotide sequences of genes carried by individuals. So, too, are the managers of the Human Genome Project, which will be a prolific source of information about the general and also the detailed architecture of the human genome. With good planning and much hard work, there will be a comprehensive listing of all functioning human genes and information about the ways in which they can vary, either harmlessly or otherwise, from one individual to another. No doubt there will develop alongside the project a flourishing trade in the diagnosis of specified genetic defects (already possible in the growing number of cases in which suitable probes have been identified). The issue is whether information relating to individuals should be disclosed to outside parties — employers, insurance companies or even intended spouses, for example.

The simple answer is that there should be no disclosure. The rules that bind physicians not to disclose information about their patients to outsiders, however vivid outsiders' interests, should apply equally strictly to those who sequence genes. Conyers's bill, whatever happens to it in the US Congress, will serve the useful purpose of emphasizing that point, but it may well be redundant even as the law now stands. Yet those who are cheering on the bill in the belief that its enactment would avoid some awkward social problems are probably mistaken.

Insurance is an important case. Insurance companies already require those applying for life insurance to answer questions about their medical backgrounds and their occupations, requiring honest answers on pain of forfeiture of benefits (not to mention premiums). If, as is probable, the Human Genome Project makes possible the more certain diagnosis of, say, the genetic determinants of heart disease, what will there be to prevent an insurance company from requiring that applicants should undergo the appropriate diagnostic test? In principle, that would not differ from the present requirement that some applicants for insurance should be examined medically before a policy is written. The practice could not be forbidden in the case of genetic information yet continued with older technology without becoming unconstitutional.

Nor would it be equitable. Just as non-smokers would have cause for complaint if insurance companies were required to offer the same terms to cigarette-smokers as to themselves, people free of specified genetic defects would rightly complain if those carrying defective and potentially damaging genes were knowingly offered insurance on standard terms. So, at some stage, genetic sequencing information is bound to become an issue. Moreover, mere reticence will not avoid the obvious untoward consequences, but will rather lose the social benefits that will arise if and when people have a better understanding of the human genetic constitution and its variations (including an appreciation that the overwhelming proportion are harmless variations).

That is why blanket legislation will not defend people against insurance companies. But people (and the new technology) need defending by a requirement that it should not be incumbent on a person to commission an investigation of his or her genetic constitution before applying for insurance, and that, once insured, he or she should not be disqualified because unwelcome information afterwards comes to light. Then what should happen to those who, perhaps because of some family investigation, are already known to be carrying an uninsurable gene? Why should people be penalized for bad luck is the question asked? The remedy, not the answer, is that congenital bad luck is a communal responsibility — a principle only slowly being accepted in the United States. □

Financial realities hit US science budget

■ Grants may be cut by 50 per cent
■ Space missions face cancellation

Washington

THE optimism of last January's White House budget request for science has finally given way before the realities of the gigantic government deficit and economic slowdown. The 15 per cent boost in major scientific research areas announced nine months ago is now on its way down, reaching 12 per cent in the latest budget figures available this week. But as budget negotiators make a last-ditch effort to bring the US deficit under control, science budgets could still be cut by some 30 per cent across the board.

1991 US SCIENCE BUDGET ($ million)

	1990 Budget	1991 Request	House*	Senate*
NIH	7,277	7,623	8,318	8,003
NSF	2,080	2,383	2,337	2,360
NASA	12,221	15,125	14,296	13,450
DOE (R&D)	3,283	3,359	3,976	4,019

* Budgets approved by congressional subcommittees.

Although congressional support of science has been stronger this year than many had expected, the estimated $150,000 million national deficit (excluding the still unknown — and probably massive — cost of rescuing the crumbling savings and loan industry) leaves little room for magnanimity in budget negotiations.

All federal agencies have been asked to make provisional plans for a 32.4 per cent reduction, the amount required to bring the 1991 budget deficit under the ceiling prescribed by the Gramm–Rudman–Hollings deficit-reduction act. Unless Congress changes the law or reaches some other compromise in the next week, Gramm–Rudman takes effect on 1 October. Federal agencies were forced to absorb Gramm–Rudman cuts for almost two months last year while congressional negotiators hammered out a budget agreement that met the deficit ceilings.

Assuming that Congress moves no more quickly this year (it seems likely that legislators will wait until after the November elections to make unpopular budget cuts), federal science agencies are preparing to make draconian funding reductions on the first of the month. The National Science Foundation (NSF) has warned funding recipients that existing grants will be cut by 50 per cent from 1 October, to allow some new grants to be awarded. "Unless there's some miracle, a 50 per cent cut seems likely", says NSF budget officer Joel Whitter. A year-long Gramm–Rudman sequestration (admittedly unlikely) would mean 21,000 fewer scientists and students supported by NSF grants.

At the National Institutes of Health (NIH), budget officials are also preparing to make difficult compromises. Latest figures suggest that existing grants would be reduced by around 30 per cent in dollar amount in the event of sequestration. The number of new and competing grants will be reduced by about 70 per cent while Gramm–Rudman cuts are in effect. If Congress delays in reaching a budget compromise, "we are talking in terms of a significant reduction in existing grants to allow for new ones", says one NIH budget officer.

Department of Energy (DOE) research facilities are likely to be another victim of austerity. Protracted sequestration would stop construction of the Continuous Electron Beam Accelerator Facility and halt the beginning of the Superconducting Supercollider and the Relativistic Heavy Ion Collider. Existing accelerator facilities such as Fermilab and the Stanford Linear Accelerator Centre would find their operating budgets cut by at least half.

Congressional priorities

Although the budget negotiations make bottom-line figures necessarily soft, the appropriations process is still an accurate gauge of Congress' science priorities, and a good indicator of cuts to come. Last week, appropriations subcommittees passed the Senate's versions of the budgets for the National Aeronautics and Space Administration (NASA), NSF and NIH. The bills join the already completed House measures and wait only for the conclusion of the budget negotiations before their differences can be reconciled in conference and final appropriations voted on.

The biggest surprises lie mostly at NASA, where the Senate joined the House in eliminating almost all funding for the Moon/Mars initiative, for which the president had requested $300 million.

The Senate subcommittee also cut $864 million (35 per cent) from the space station, more than four times the House cut and a significant blow to the programme.

Although the space station may be able to weather the loss, some smaller projects face outright cancellation. The CRAF/Cassini cometary exploration probes lost $50 million from their $148 million request. Because work must begin soon on the spacecraft if they are to be ready in time for a mid-1990s launch window (when the intended comets will be within reach), even a one-year funding delay could cause NASA to abandon parts of the project. As the European Space Agency has already committed itself to the Cassini mission, CRAF appears to be the odd man out. "If the end result of the $50 million cut is that one of them has to be dropped, it will be CRAF", says NASA project manager Henry Brenton.

The Senate also shifted funding within the Earth Observation System programme from large satellites to smaller ones, and restored funding for an existing search for extraterrestrial life that the House bill had derisively deleted. The crippled Hubble space telescope was given $30 million in extra funding to help speed corrective measures.

Among the federal science agencies, NSF fared best last week, as the Senate appropriations subcommittee approved a 14 per cent increase to $2,360 million, a figure that lies close to the presidential request. Like the House, however, the Senate eliminated funds for the Laser Interferometer Gravitational Wave Detector and emphasized education increases over research.

No crisis at NIH?

In last week's NIH bill, the Senate differed from the House in granting the full administration request for the genome project ($108 million; the House had cut that to $66 million [see *Nature* **346**, 309; 26 July 1990]). With the exception of AIDS funding, which the Senate reduced by some $300 million, overall NIH funding in both bills represents a 10 per cent increase over last year.

Like the House bill, the Senate report is expected to include controversial language instructing NIH to reform their grant process. Among the directives, the House bill calls for NIH to increase the number of grants they issue to 6,000 (compared to 4,600 new grants this year) by reducing the length and the rate of growth of the average award, and favouring institutions with lower financial overhead, or "indirect costs". Claiming that the "crisis" in health research "has been overstated", the House bill offers the reforms as a way to "bring more stability and predictability to NIH's current fiscal situation". Although Senate staff say their language will be much more "muted", it appears likely that reforms of one sort or another will become law. NIH have formed ten committees to suggest how the institutes should implement the congressional language. Their reports are due by mid-October.

Christopher Anderson

UK UNIVERSITY RESEARCH

More suitors from abroad

London

In the latest in a series of long-term investments by overseas drug companies in UK university-based research, Eisai, an expanding Japanese company, last week announced that it will spend £50 million over the next 15 years to set up a new neurosciences research centre at University College London (UCL).

The move has been welcomed by the UK scientific community but at the same time has reawakened criticism of British companies, which show little sign of emulating their foreign competitors' large-scale investment in universities.

The centre will be housed in a new building, to open in 1992, concentrating on basic research into the factors underlying diseases of the central nervous system such as Alzheimer's and Parkinson's. Short-term commercial benefits are unlikely, but any patent rights will be held by Eisai. The centre's 30 staff will be Eisai employees, and so will need approval before they can publish research data. But Derek Roberts, provost of University College London, does not expect any problems, because Eisai has a good record of allowing employees freedom to publish.

Roberts points out that Eisai chose to site the new centre at UCL, rather than at a less expensive 'green field' site, because it wishes to gain from the exchange of ideas with other academics on the campus. A restrictive policy of commercial confidentiality would soon subvert this aim.

Professor Denis Noble, president of the academic pressure group Save British Science, says the Eisai investment is part of a welcome trend towards closer relationships between university researchers and overseas companies. But he says Eisai's "enlightened" attitude is not shared by British companies, which prefer to use universities strictly for contract research aimed at specific and short-term goals.

Roberts says that UCL finds it "singularly difficult to persuade British companies to put money into bricks and mortar". Little money for new buildings is forthcoming from government, through the Universities Funding Council, so the best option for universities wishing to expand their research facilities is to interest overseas companies. UCL already hosts a pharmacology research centre funded by the Swiss company Sandoz, which opened in 1985. Sandoz leases laboratory space from the college, providing money that has allowed the chemistry department to move to a new building.

Professor David Smith, from the University of Oxford's pharmacology department, another beneficiary of major overseas investment, is also disappointed with the response of British industry. In the mid-1980s, faced with declining government funding, his department began looking for increased industrial sponsorship. But Smith says that of 23 companies that attended a workshop in 1986 to hear about the department's work, only two were British. The Oxford department eventually signed a seven-year £20 million agreement with the US company Squibb (now Bristol-Myers-Squibb) in 1987, to finance a new building for the department and run a number of research projects. Under the Oxford–Squibb arrangement, researchers are not Squibb employees. Squibb files for any patents on the results of research, but the university receives a royalty on any commercial gains.

UK pharmaceutical companies argue that they already contribute to the British universities through the tax system, and point out that companies — both British and foreign — like to be seen to be supporting local research when they are operating in overseas markets. As an example they note that the British company Glaxo last year provided £15 million to set up a neurosciences centre at the National University of Singapore for research into dementia and Parkinson's disease.

For overseas companies, there is also a direct financial advantage that may offset investment in UK universities: drug companies may charge the National Health Service more for their products if they have substantial research activity in the United Kingdom. British companies have no such incentive to invest in university research.

But there does seem to be some genuine dissatisfaction within the British pharmaceutical industry over arrangements for research in UK universities, John Griffin, director of the Association of British Pharmaceutical Industry, last week told the British Pharmaceutical Conference in Cardiff that universities are charging inflated overheads on research contracts to subsidize 'lame duck' departments.

Peter Aldhous

US CANCER EPIDEMIOLOGY

No Sellafield effect in US study?

Washington

A two-year study to be released this week has found no increased incidence in cancer mortality around nuclear facilities in 107 US counties, but researchers warn that the resolution of the data may be too coarse to draw firm conclusions.

The study, conducted by a team at the National Cancer Institute (NCI) led by John Boice, was begun in 1987, after a similar British study published in this journal suggested that there might be a higher incidence of childhood leukaemia near nuclear installations (see *Nature* 329, 499; 1987). Subsequent studies, including one published this year that provided evidence of a cluster of cases of childhood leukaemia in the vicinity of the Sellafield nuclear plant (see *Nature* 343, 679; 1990), have raised more questions than they have answered.

The NCI study seems likely to be no exception. "From the data at hand, there was no convincing evidence of any increased risk of death from any of the cancers we surveyed due to living near nuclear facilities", Boice stated last week. But he also said that the size of the areas from which data were collected may be too large to detect small pockets of cancer immediately around nuclear plants.

In the 1987 British study, David Forman and colleagues from the Imperial Cancer Research Fund Cancer Epidemiology and Clinical Trials Unit, Oxford, used local authority areas (LAAs) as the units of geographic location of the surveyed populations. Forman's team used only LAAs with at least one-third of their population living within 10 miles of a nuclear facility. Many of the LAAs had two-thirds of their population living within six miles of the installation.

The US counties used in the NCI study are much larger. The NCI team used county mortality records, rather than those located by zip code (which tend to represent much smaller areas), because they were the only complete record to which the researchers had access at the time. "We recognized that it was a problem, but [counties] were the smallest unit for which death data was available", says team member B.J. Stone. "If we had had a choice, we would have gone to census-tracked data." For childhood leukaemia, the NCI study found that mortality risks were slightly higher before the start-up of a nuclear plant in the county than after. For other forms of childhood cancer, the reverse was true. But in both cases, the change in risk is so small (1.08 to 1.03 in the first case, 0.94 to 0.99 in the second) and the counties were so large, that the researchers were not able to attribute it to the presence of the facility. The conclusions of the report recommend a more detailed study.

Although no "patterns jump out at you", says Stone, "there are some plants that are significantly high and some that are significantly low. You do have this feeling that you should go back to certain areas [and do a more detailed analysis] because you feel uneasy." Given the uncertainties in the NCI report and the interest of Senator Edward Kennedy, one of the chief backers of the study, a further project appears likely. Christopher Anderson

INTERNATIONAL INSTITUTES

Radical computing in Japan

Tokyo

NEURAL, biological and optical computing are among the radical forms of computing to be taken up by a new international institute to be set up in Japan with the backing of the private sector. The institute is the work of the Japan Technology Transfer Association (JTTAS), a non-profit organization affiliated with the Ministry of International Trade and Industry (MITI), and has already won support from a large number of Japanese companies.

The aims of the International Institute of Novel Computing (IINC), as it will be named, appear to overlap with the so-called sixth-generation computer project which MITI is expected to launch in 1992 (see *Nature* **345**, 279; 1990). But backers of IINC say the two projects are not directly related.

JTTAS, which is affiliated with MITI's Agency of Industrial Science and Technology (AIST) but funded almost entirely by private industry, began soliciting contributions last April from private companies for a feasibility study for IINC. The association's activities initially angered MITI's machinery and information industries bureau which is promoting the sixth generation project. According to Taizo Nishikawa of the bureau's industrial electronics division, JTTAS was giving the impression that the IINC project is officially backed by MITI when in fact it is not (part of the problem was that JTTAS is affiliated to a different section of MITI).

But Hideo Aiso of Keio University, the chairman of IINC, says that there is no real conflict with MITI. The two projects, he says, are like "two vectors" going in different directions although it is possible they could merge in the future. (Oddly enough, many of the academic advisers for IINC are also advising MITI on its project).

Aiso says that previous national computing projects, such as the fifth-generation computer project, have tended to set their sights on making a particular piece of hardware, something which is necessary to get backing from the Ministry of Finance. But IINC supporters, such as Shunichi Amari of Tokyo University, one of Japan's leading experts on neural computing, see a need for a more flexible approach that will allow more basic research and may also encourage the participation of foreign researchers. They think the best way to achieve that goal is to avoid government funding at least in the initial phase of the project.

Nine companies, including Sanyo, Hitachi, Nippon Steel Corporation, Mitsubishi Electric and Nissan, have already agreed to contribute ¥1.8 million ($13,000) a year towards the IINC feasibility study, which will last "about two years", according to Aiso. And many more, including Toyota, NEC, IBM Japan, Honda, Fujitsu, Sharp, Sony, Tokyo Gas Co., Japan Railways and Nippon Telegraph and Telephone Corporation are shortly expected to join, says Isao Idota, managing director of JTTAS. JTTAS's final target is 100 companies.

Twelve subcommittees each with about 20 academics and industrialists have been established for the feasibility study and they have already begun regular meetings. The subcommitees, headed by leading academics in each field, cover neurocomputing, biocomputing, optical computing and parallel computing as well as several other fields. Thus, the research areas overlap in many respects with the sixth generation project. But Aiso does not expect all of these areas to be covered when the institute finally opens.

Each subcommittee has members from the companies supporting IINC and also several foreign "observers" who will be kept informed of subcommittee deliberations and will be tapped for advice. Among them are Terence Sejnowski of the Salk Institute in the United States and Harold Szu of NASA.

Aiso and Amari say they are determined to make the institute truly international. There have been several recent attempts by MITI to set up so-called "international" institutes but they have failed to draw support from foreign companies and have been unable to recruit significant numbers of foreign researchers.

Aiso and Amari say that the solution may be to establish an institute in the United States and Europe first and only later set one up in Japan.

David Swinbanks

ENVIRONMENTAL RESEARCH

European body gearing up

London

JOHN Lake, formerly head of science with the UK Agricultural and Food Research Council, is to become the first director of the European Environmental Research Organization (EERO). EERO was conceived as an analogue to the European Molecular Biology Organization, to support basic research in environmental science. It has about 40 members, all eminent European environmental scientists, and a small permanent office in Wageningen in the Netherlands.

Lake says that EERO-supported research must be led by the opportunity of making new scientific discoveries, rather than responding to political demands. Applications for EERO's first round of up to 15 fellowships have just closed. Lake hopes to increase EERO's current annual budget of 2.5 million ECU (1 ECU = £0.70) by "several fold" over the next few years, raising funds from European governments and charitable foundations. The Dutch government and the Volkswagen Foundation have already given support.

Peter Aldhous

UK RESEARCH

Call for increased polytechnic spending

London

THE UK Polytechnics and Colleges Funding Council (PCFC) should increase its spending on research from the current £30 million a year to £58 million in 1991–92, according to the report from a PCFC-appointed committee, released today (20 September).

The research committee was set up in April last year by the newly formed PCFC to examine the future role of research in the polytechnic and college sector. Its chairman, Oscar Roith, chief scientist at the UK Department of Trade and Industry, says that many committee members were at first sceptical of the value of polytechnic research. But he claims that all are now convinced that research is "a key component" of polytechnics and colleges.

A survey by an independent team of consultants found that the sector's external research income exceeds that from PCFC by 1.7 to 1. The committee believes that increasing PCFC's spending on research would attract more research funds from industry and the research councils. Polytechnics and colleges received only £6.7 million in research council grants in 1988–89 (about 4 per cent of the councils' annual research grant expenditure).

To increase this, PCFC is to set up a working party to look at the polytechnics' relationship with the research councils. Roith says that polytechnic researchers often do not apply to research councils because they believe university applicants are favoured. But Roith says that the research councils assured his committee that this was not the case.

Nevertheless, universities do have a real financial advantage. Research council grants provide only for the direct costs of research, but polytechnics receive no government funds for research overheads. Universities, by contrast, benefit from the 'dual support' system, where research council grants are supplemented by the research component of the Universities Funding Council (UFC) block grants. In 1988–89, universities spent about £770 million of their UFC income on research.

Peter Aldhous

VACCINE RESEARCH

WHO calls the shots

Munich

THE World Health Organization (WHO) and several other international organizations will next week launch an ambitious campaign for universal vaccination of children against diseases such as polio, tetanus and measles at the 'Children's Summit' in New York City. The creators of the 'Children's Vaccine Initiative' say that this will be the first time a broad network of agencies and world leaders will have given their support to the cause of better vaccines, which are the most cost-effective way of saving lives in the developing world.

WHO, the United Nations Development Programme (UNDP) and the United Nations Children's Fund (UNICEF) have joined forces to back an effort that they hope will attract a minimum of $15 million a year over ten years for vaccine research and development. An expected 70 heads of state, including Soviet leader Mikhail Gorbachev and US President George Bush, will pledge their countries' support at the summit on 29–30 September.

The 'New York Declaration' sets goals of reducing the number of doses of vaccine required for immunization; combining many antigens in a single vaccine; making existing vaccines more heat-stable and affordable; and developing new vaccines against, for example, diarrhoea and acute respiratory infections.

An earlier goal — that of developing a single-dose, preferably oral "children's vaccine" that could immunize children against all endemic diseases — was dropped at a pre-summit meeting of WHO, UNDP and UNICEF experts in New York on 9–10 September.

According to Friedrich Deinhardt of the University of Munich, chairman of the WHO Scientific Advisory Group of Experts (SAGE), it was thought impossible in the near future to use a single vaccine carrier, such as a vaccinia virus, to carry antigens against as many as a dozen diseases. But, says Deinhardt, the use of existing techniques will allow the development of combination single-dose vaccines that would reduce the number of vaccinations necessary to immunize each child, thereby increasing effectiveness and reducing cost.

WHO has succeeded beyond all expectations since it began vaccinating children in 1974 under the Expanded Programme on Immunization. Whereas just 5 per cent of children in the developing world were being vaccinated in 1974, the programme now vaccinates 66–81 per cent of children against deadly diseases such as measles and polio.

Nevertheless, advocates of the Children's Vaccine Initiative are not satisfied with the results of the Expanded Programme on Immunization. "Not a single new vaccine has been introduced in the developing world" since the programme began, says Barry Bloom of the Albert Einstein College of Medicine in New York.

And the vaccination programme is still plagued by technical problems, especially that of keeping the vaccines cold from the time they leave the manufacturer to the time they arrive at their destination, often in the tropics.

Even after next week's anticipated rousing start, the initiative will face two large challenges. For one, persuading researchers to work on vaccines will not be easy. "No one gives a prize for making polio vaccine more heat-stable", says Deinhardt. But Bloom, more of an optimist, says that the scientific problems might be interesting enough to catch the attention of researchers. "It's not just pickling a new bug, grinding it up and jabbing it into people", Bloom says. Instead, the research might require "using genetic engineering to make the vaccines less sensitive to heat", he says, "a project which is not trivial." The other challenge is raising money. Even though $150 million sounds an impressive amount, Deinhardt says it is little more than "seed money" to get researchers and companies started on vaccine research. Developing a modern vaccine can cost as much as $20–$100 million, including expensive animal and human trials. According to Jim Sherry of UNICEF, US industry is ready to play a large part in developing better vaccines, but only if someone else pays the bill.

One likely target for the Children's Vaccine Initiative fund-raisers will be the US government. The list of supporters of the WHO Programme for Vaccine Development, an adjunct to the Expanded Programme on Immunization, does not include the United States but does include Australia, Italy, Japan, Norway, Sweden, Switzerland, the Rockefeller Foundation and UNDP.

US Senator Bill Bradley (Democrat, New Jersey) recently introduced a bill into the Senate to support the Children's Vaccine Initiative with as much as $125 million a year, although the bill has little chance of becoming law. Nevertheless, Sherry believes that finding support will become easier for researchers who put forward good proposals to US agencies such as the National Institutes of Health.

Steven Dickman

GLOBAL WARMING

France comes cleaner than clean

Paris

FRANCE last week joined the list of countries preparing to take action to reduce greenhouse gas emissions. In a memorandum addressed to the Presidency and the Commission of the European Communities, the French secretary of state for the environment, Brice Lalonde, has declared that France will reduce levels of carbon dioxide emissions to "less than 2 tonnes per capita per year" in the next 25 years. The present emission level of carbon dioxide in France is about 2.3 tonnes per capita per year.

According to a spokesman at the ministry of foreign affairs in Paris, the announcement has been made to help advance negotiations at the ministerial meeting that will follow the Second World Climate Conference to be held in Geneva at the end of October.

Within the environment ministry — which has just seen its annual budget almost double — a spokesman described the French proposal as "ambitious". But France is not a major producer of greenhouse gases, contributing only 1.7 per cent of world carbon dioxide emissions and about 2 per cent of all greenhouse gas emissions. Neighbouring West Germany contributes 3.3 per cent and the United States, 23 per cent of world carbon dioxide emissions (1989 OECD figures).

Energy production in France causes relatively little atmospheric pollution. France has few fossil fuel resources and, following the oil crisis of the 1970s, successive governments backed expansion of the nuclear energy programme. Now only about 9 per cent of the nation's energy needs are met from coal, compared with 32 per cent in Britain and 27 per cent in West Germany. By contrast, some 30 per cent comes from nuclear generators, compared with 7 per cent in Britain and less than 12 per cent in West Germany.

Over the past decade, France's reliance on coal has dropped by 40 per cent, while nuclear energy production has more than tripled.

Now, with the nuclear industry entering a period of decline, the Gulf war could have provoked new investment. But the industry minister, Roger Faroux, has said that he intends only to continue the present programme of reactor construction and has no plans for expansion. Instead, a more or less shelved programme to develop alternative energy sources will be revived, while new measures have been announced to promote energy saving. Of these, extra money will be available to develop a 'clean' car. Transport accounts for almost 40 per cent of the nation's carbon dioxide emissions.

Peter Coles

US UNIVERSITY OVERHEADS

Stanford argues over costs

Boston

WITH the highest research overhead costs in the United States, Stanford University and its outspoken president, Donald Kennedy, have long been at the centre of the tortuous debate over the standards by which Stanford and other universities calculate overhead charges to the government when they receive federal research grants. Last week, in a new twist to the story, the university revealed that three separate investigations into the university's practices for recovering overhead costs associated with federally-sponsored research are now under way. In addition to Stanford's own "internal audit", branches of Congress and the Defense Department will review the school's practices, with millions of dollars of research funds potentially at stake.

The most explosive investigation is being led by the Office of Naval Research (ONR), the branch of the Defense Department responsible for negotiating the overhead cost rates with Stanford on behalf of all federal agencies. An internal memorandum from a field representative of the ONR that came to light last week accuses Stanford officials of possible "abuse" and "distortion" in their calculation of overhead costs. The letter concludes that "Stanford engaged in fraudulent acts, and, at a minimum, false statements and false claims". Curiously, senior officials at ONR say that they never received the memorandum, which is dated 6 March 1990. Both Stanford and ONR officials claim to have learned of the accusations only recently when the memorandum surfaced as part of a request under the Freedom of Information Act from a local California newspaper. Because of the seriousness of the accusations, ONR has launched a full-scale investigation.

The issue is complex and divisive well beyond the specific confines of the ONR investigation, however. Congressman John Dingell, whose congressional subcommittee has also now begun to investigate the overhead issue, informed Kennedy last month that "escalating overhead rates" at Stanford and other universities have prompted his committee's investigation, which will begin with Stanford but will ultimately review practices at other US universities.

Congressional staff members say that the investigation seeks to determine "exactly what the government is paying for" when it allots hundreds of millions of dollars in overhead charges. Stanford alone, for instance, received some $91 million in overhead costs last year.

Not surprisingly, university administrators, facing rising energy and operating costs, are conscious that the fate of their institutions is bound up in the overhead issue. As for specific charges against Stanford, Kennedy stated last week that university staff responsible for indirect cost matters "enjoy excellent reputations in their professions" and will "cooperate fully with all investigations". But he said that university officials will not comment on the allegations until the investigations are complete.

Stanford faculty members protested strongly last spring that Stanford's rising overhead cost rates — now 78 per cent — were affecting their ability to attract federal grants. Competing researchers, they maintained, were "underselling" them because competitors could conduct the same research at other institutions for less total cost to the government.

Under the current system, universities are allowed to recover overhead, or indirect costs, associated with their researchers' grant proposals through a complex set of guidelines. These indirect costs are computed as a percentage of a modified version of the total direct costs associated with a research project; they can include charges for the university's costs of utilities, building maintenance, the depreciation of new facilities, library use and other administrative costs. Thus, if a Stanford researcher's federal grant has a "modified total direct cost" (for researchers' salaries and the like) of $100,000, the federal agency dispensing the grant will be asked to pay $178,000 to cover Stanford's share of indirect costs. By comparison, indirect cost rates at MIT, Yale and Columbia are 62 per cent, 68 per cent, and 74 per cent respectively.

As high as those indirect cost rates sound, Larry Horton, Stanford's associate vice president and director of government relations, emphasizes that the percentages themselves are misleading. The rates, he points out, are applied only to a certain portion of a given federal research grant. Thus, Horton maintains that, of total federal funds allotted to Stanford for research, the government actually paid some 43 per cent in overhead charges. However, many researchers still believe that the indirect cost rates are out of control, and government officials complain that many private foundations, such as the American Cancer Society, refuse to pay overhead costs, leaving federal funders to shoulder a disproportionate share.

The controversy surrounding the issue of indirect costs is a longstanding one. Nonetheless, all involved in the current controversy at Stanford predict that the newest round of investigations will once again bring the issue prominently before the public and could provoke new limitations on the indirect costs deemed admissible from universities to the federal government.

Seth Shulman

PATENT DISPUTES

GE sees patent storm rising

Washington

WHO first thought that isotopically pure diamonds might be the best room-temperature heat-conductors ever created, with a host of possible applications in the electronics industry? General Electric (GE) took credit for the manufacture of the first such pure diamonds at a press conference earlier this year. But Russell Seitz, a Cambridge (Massachusetts) materials scientist, claims that he predicted the high room-temperature thermal conductivity of isotope-enhanced diamonds in a 1975 patent, and told GE researchers about it in 1986.

Most scientists might turn to their lawyer when they suspect someone has borrowed their ideas, but Seitz has instead enlisted the aid of bestselling techno-thriller author Tom Clancy, who used Seitz's predictions about diamonds in *The Cardinal of the Kremlin*, and a host of famous scientists to persuade the GE board of directors to give him some credit for the work.

In a letter to be sent to GE this week (with a covering letter from Clancy), physicists Philip Morrison and Richard Wilson from Massachusetts Institute of Technology and Harvard respectively, join such notable researchers as artificial intelligence pioneer Marvin Minsky and former presidential science adviser George Keyworth in arguing Seitz's case. "In denying Seitz's seminal role in the development of mono-isotopic diamond . . . , GE has not only failed to live up to its own reputation as an ethical firm, but has transgressed the norms of professional scientific conduct", the letter charges.

GE board members have not yet seen the letter but others at GE reject Seitz's claim. Walter Robb, senior vice president for corporate research and development, says that Seitz is one among many who speculated on the effect. "I suppose in the press release and technical article we could have listed all 10 or 20 authors that commented on this effect . . . if we had mentioned a number of them he (Seitz) probably would not have been in the list".

Seitz says that he described the effect discovered by GE at a 1987 talk attended by GE scientists. But GE scientist William Banholzer says that "If he did make such a prediction, it was nothing more than speculation . . . He's had three years to publish it in a refereed journal. Where is it?"

Seitz and GE researchers will have a chance to reconcile their differences next week at a Washington, DC, scientific meeting at which both will present papers. Clancy will be giving an after-dinner speech and Seitz hopes it will be one "congratulating GE" on giving recognition to his contribution.

Christopher Anderson & Alun Anderson

US SCIENCE AGENCIES

Top names at last

Washington

AFTER more than a year's vacancy in the top post at the US National Institutes of Health (NIH) and a protracted search for a new US National Science Foundation (NSF) director, the administration last week finally revealed its choices.

Bernadine Healy, a nationally known cardiologist, is to head NIH, and Walter Massey, vice-president for research at the University of Chicago and Argonne National Laboratory, will run NSF, assuming that there are no snags in the congressional confirmation process.

Healy has criticized NIH for underrepresenting women in clinical trials, paying too little attention to women's health issues and neglecting women researchers when selecting managers.

Massey, a physicist, is a member of the National Science Board and is vice president of the American Physical Society. He has recently taken a special interest in issues of technology transfer from research to industry. Christopher Anderson

HUBBLE SPACE TELESCOPE

Upside down rod

Washington

AN investigative team assembled by the National Aeronautics and Space Administration (NASA) to find the cause of the Hubble Space Telescope's optical distortions last week fingered the culprit.

A measuring instrument known as a metering rod may have been used upside down, aggravating a separate problem in which reflections from a cap placed at the end of the rod sent false signals that confused the technicians shaping the mirror, the team said.

"Aspects of th[is] scenario have been reproduced with an interferometer and metering bar/end cap simulations, and the end results indicate that the scenario as described is probable", team leader Lew Allen, director of the Jet Propulsion Laboratory, said in a statement.

The metering rod is used to set precisely the distance between elements of an optical device known as a null corrector that was used to help shape Hubble's main mirror. A 1.3 mm error appears to have arisen when technicians fitted an end cap with a pinhole at a position 1.3 mm beyond the end of the solid metering rod, to ensure that the reference beam was reflected off the very centre of the rod's end. However, the end cap was itself shiny and the light beam seems to have been reflected off the cap, rather than travelling through the pinhole.

The fact that the metering bar seems to have been used upside down, with its intended reflective end away from the light beam, only made the situation worse.

Christopher Anderson

HUMAN GENOME SEQUENCING

Japan gets its act together

Tokyo

THE Japanese government is at last trying to put together into one coordinated whole its various efforts to contribute to international plans to sequence the human genome. Alongside an expansion in funding for genome-related research, the Council of Science and Technology, Japan's principal science policy-making body, will try this week to harmonize the competing activities of various government ministries and agencies.

Japan's financial commitment to research on the human genome has so far been small, and has provoked complaints from James Watson, director of the US National Institutes of Health Center for Genome Research (see *Nature* **342**, 463; 1989). Budget increases now under way will still leave Japan with a small contribution in relation to its gross national product, but nevertheless represents a small victory for Japanese scientists.

The Ministry of Education, Science and Culture (MESC) is to boost funding for grants from ¥280 million to ¥500 million ($3.6 million) per year, following the completion of a two-year preparatory study put together by Kenichi Matsubara of Osaka University. The ministry has also requested ¥850 million ($6.2 million) over five years to establish a new Genome Analysis Centre at the Institute of Medical Science at Tokyo University. But the centre, which MESC hopes eventually will have a staff of about 20 people, still faces a difficult future because of the freeze on the appointment of new government employees. MESC's plans for the centre are in fact extremely ambitious, as the ¥850 million is only the beginning of a much larger expenditure, and MESC wants to supplement the activities with new posts at universities throughout Japan.

A further problem, according to Matsubara, may be a conflict with the Science and Technology Agency (STA)'s plans to support related work at the Institute of Physical and Chemical Research (RIKEN). Masato Chijiya, director of STA's Life Science Division, says that such an overlap is one of things that will have to be sorted out by the Council of Science and Technology and a committee of ten scientists headed by Wataru Mori, former president of Tokyo University, that will be appointed by the council on 21 September.

STA plans to expand its budget only slightly — by about ¥ 100 million ($1 million) — for genome-related research next year. A total of ¥542 million ($4 million) has been requested for RIKEN, of which ¥303 million will go towards development of automatic DNA sequencing machines and to DNA sequencing. The remainder goes to a gene bank which is already under development at RIKEN's Life Science Centre in Tsukuba.

Other ministries are also climbing aboard the genome bandwagon. The Ministry of Health and Welfare is requesting ¥400 million, up from ¥200 million this year, for a project to analyse and sequence oncogenes. And the Ministry of Agriculture, Forestry and Fisheries is requesting ¥610 million for fiscal year 1991 to begin sequencing the rice genome. For this project, which is expected to last seven years, ¥155 million is allocated to DNA sequencing and research to be carried out largely at the ministry's National Institute of Agrobiological Resources in Tsukuba, ¥326 million is for mapping that will be farmed out to private companies, ¥100 million is for equipment and facilities, and ¥16 million will go to study of the establishment of yet another DNA bank.

Still to join the fray is the powerful Ministry of International Trade and Industry (MITI). Michio Oishi of Tokyo University is trying to win support from MITI and private industry. Japanese companies do not see much of market in the human genome project itself but are interested in the possibility of developing diagnostic machines for the medical market, Oishi says. It will probably be some time before MITI makes a move but if it does, Oishi is confident that its budget will be larger than that of other ministries.

All of the budget requests are subject to negotiation with the Ministry of Finance but they are unlikely to undergo drastic change. If granted in full, Japan's total government budget for human genome research will still be less than a quarter that of the United States (but well above that of many European countries).

Despite the extra money, Matsubara sees no sign of Japanese funds for the Human Genome Organization (HUGO), which will coordinate the international research effort. The Wellcome Trust in the United Kingdom and the Howard Hughes Foundation in the United States have contributed more than a million dollars to HUGO and one of Watson's main criticisms was directed at Japan's failure to contribute to that organization.

Very restrictive laws on the establishment of foundations in Japan are effectively blocking Japan's ability to contribute to HUGO, according to Matsubara. To establish a foundation, ¥200–¥300 million ($1.5–$2.2 million) must first be raised, which actually means raising twice as much because donations initially are not tax free. Then the foundation must operate for several years before it can win tax-free status.

David Swinbanks

GENETIC SCREENING

Law to keep labels off genes

Washington

FEARS that discrimination against those at risk of developing genetic disorders will be a malign result of increasing knowledge about the human genome have prompted a bill introduced last week by Representative John Conyers (Democrat, Michigan).

The Human Genome Privacy Act, the first of its kind anywhere, promises to give individuals the right to privacy on information about their genetic make-up. Discrimination based on an individual's genetic diagnosis and screening records would be prohibited in areas such as employment, insurance and education.

Cases of 'genetic discrimination' have already been documented. According to Paul Billings, vice chairman of the department of medicine at the Pacific Presbyterian Hospital in San Francisco and a prominent supporter of the Privacy Act, individuals have been denied health insurance and employment because they were known from genetic evidence to have disorders such as Charcot-Marie-Tooth muscular atrophy and the neurodegenerative disorder Friedrich's Ataxia.

Asymptomatic carriers of recessively inherited disorders including sickle-cell anaemia and Gaucher disease, a disorder of lipid metabolism, have also been refused employment because of misguided fears that they themselves were susceptible to the disease.

The new act would prevent the disclosure of genetic information by government agencies, contractors or grant-recipients without an individual's written consent. Exceptions would be made in cases of medical emergency and criminal investigation. Conyers, chairman of the government operations committee, has won widespread backing within the scientific and religious communities for the legislation and expects bipartisan support in Congress.

Long-time adversaries Jeremy Rifkin, president of the Foundation on Economic Trends, and W. French Anderson, a leading researcher into gene therapy at the National Institutes of Health (NIH), both find themselves backing the initiative.

Rifkin hopes the act will serve as a model for similar legislation soon to be introduced in about 20 countries, mainly in Europe. Although the proposed US legislation applies only to government agencies and their affiliates, "it is important that government establishes the standard by which private industry will then respond", Rifkin said last week at a press conference held to launch the act.

Anderson was also expected to speak at last week's press conference, but withdrew at the last minute because the Department of Health and Human Services "denied me permission to go as an NIH scientist", he said. Nevertheless, Anderson affirmed that he "strongly supports the concept behind the bill" and its introduction as a "means of initiating public discussion".

Billings says that "the variability of genetic disorders and the fact that some of them are not particularly severe are not appreciated by the general public or by the people who are making policy decisions for employers, insurers or even adoption agencies". Congessional hearings of the Human Genome Privacy Act will begin next spring.

Kevin Davies & Diane Gershon

EDUCATION

University of democracy

Paris

ABOUT 40 Chinese students attended last week the 'Summer University of Democracy' in Paris. For one week the students were able to hear talks on different aspects of democratic civilization given by a dozen leading academics, mostly specialists in law and economics, and Jiaqi Yan, a dissident and former adviser to the Chinese Prime Minister.

The 'university' was financed from funds raised by the French Ministry of Culture on the initiative of the Maison Chinoise pour la Democracie, which was set up in June 1989 as a focus for efforts to establish democracy in China, following the Tianenmen Square massacre.

Peter Coles

SUPERCOMPUTERS

London centre in funding row

London

ONE of Britain's three academic supercomputing centres may be forced to cut back its services, because of a dispute between the Department of Education and Science (DES) and the University of London over the centre's funding. The DES Computer Board has told the University of London that funding for the University of London Computing Centre (ULCC)'s Cray supercomputer must be reduced from almost £2 million to £1 million a year, but the university is reluctant to foot the bill. The board also wants to cut funding for the centre's smaller Amdahl computer by almost £900,000.

Together with a similar-sized centre at the University of Manchester and the larger Atlas facility at the Science and Engineering Research Council's Rutherford Appleton Laboratory (RAL), ULCC provides a national supercomputing service for the UK academic community. But ULCC currently costs DES about twice as much to run as the Manchester centre.

ULCC's actual operating costs differ little from those in Manchester — both run a large supercomputer and a second-string machine. But the Manchester centre is run as a local computing facility for the university. This means that the university can run the national service at 'marginal cost', simply charging DES for the extra staff, software and maintenance needed to supplement the local service. ULCC, by contrast, is separate from the computing centres at each of London's colleges, so the bulk of its running costs fall to DES.

Professor John Forty, chairman of the Computer Board and principal of the University of Stirling, says that ULCC's costs are "abnormally high" and must be brought down "in the national interest". But this means that the University of London must take on more of the centre's running costs or accept cutbacks. Faced with an accumulating financial deficit (predicted to rise more than £40 million by 1992–93), the university is likely to take a tough line, not least because contributing more to ULCC's running costs would set a dangerous precedent for the other national facilities hosted by London.

London's federal structure also makes it difficult to link ULCC with one of the university's other computing centres and run it as a University of London service with the national service provided at marginal cost. The other centres are attached to individual London colleges and are not provided for the university as a whole.

The Computer Board is expected to make a final decision on ULCC's future funding in October. If the University of London refuses to take more of the load, the board will consider switching some work to the other two national supercomputing centres. But Brian Davies, head of computing at RAL, says "the slack couldn't be taken up on the machine we've got". Professor Frank Sumner, director of computing at Manchester, says the Manchester machines would need upgrading to absorb extra work.

The drive to cut costs in British academic supercomputing contrasts with expanding capacity in other countries. Sumner says that British academics were "far better provided for" than their US colleagues six years ago, but investment in new facilities by the National Science Foundation means the United States is now "way ahead".

Expansion is also under way in West Germany, with the German company Siemens installing new machines in Karlsruhe, Aachen and Hanover over the next six months. German academics benefit from the desire of each of the *Länder* to host a state-of-the-art supercomputing centre, with regional government putting up half of the cost of each.

Peter Aldhous

In defence of taxonomy

In *Nature* of 16 August (Vol. 346, 602; 1990), H. T. Clifford, R. W. Rogers and M. E. Dettmann argued that taxonomists might usefully dispense with the existing massive herbarium collections. We have received many letters criticizing this view. Here we publish those received first; the remainder make many of the points below.

SIR—Clifford, Rogers and Dettmann have exaggerated the problems faced by taxonomic institutes and have misunderstood the role of herbaria. They suggest a solution that displays ignorance and a surprising lack of understanding in professional biologists.

The worry is that their solution may appeal to the uninitiated, and could be taken up by busy administrators and politicians seeking quick remedies to immediate ills. As representatives of the systematic botany community in Australia, we would like to stress that systematics is not merely an exercise in stamp collecting or a naming service for other branches of biology.

Briefly, Clifford *et al.* state that herbaria are becoming choked by ever-increasing numbers of specimens, most of which, in their opinion, have so little value that we would be better off without them; they should, the authors say, be pulped. The principle can be applied to all taxonomic collections. With touching optimism, they go on to suggest that funds and staff-time so saved would be diverted to "taxonomic research proper".

No part of Australia's flora is well known *in toto*, but we probably all know of individual species that are so well-represented that some specimens could be pruned without loss. But even if a specialist were to prune, the saving in curatorial load would be negligible. All the institutes we represent already practise some pruning and quality control of incoming material; some reserve sterile material apart until after publication of results, and disposal seems appropriate. But no case at all can be made for ditching the bulk of the collections.

The lack of understanding of the difference between written records and specimens shown by Clifford *et al.* is little short of stupefying. A description makes accessible a selection, a subset, of the total information that a specimen yields. There is no such thing as a complete description; there will be as many descriptions as there are disciplines studying that specimen, and many of them will not overlap. Yesterday we would have had descriptions of gross morphology, anatomy and palynology. Today, we have electron microscopy and biochemistry in many new and revealing facets. Tomorrow, who knows? No specimens, no information.

Today, systematic biology is being rejuvenated by new and more disciplined ways of thinking; the computer provides powerful new tools and the predictive power of the resulting classifications — the central aim of the systematist — improved. Without specimens, variation cannot be assessed. The amassed collections of ourselves and our forebears now have new potential in the urgent task of discovering, describing, naming and above all understanding the relationships and biology of the riot of life around us while it lasts. It is time to build on the resources of our collections, not to discard them untapped.

Finally, the authors confuse herbaria (the collection of dried plant specimens) with Herbaria (the institutes that care for and use them). The value and usefulness of herbaria are judged by the number of specimens, the geographical areas covered, the groups represented, the state of their curation and the proportion of 'classical' material mentioned in the literature, including types. But Herbaria are indeed judged in part by the quality of their research, in part by their attitude and accessibility to visiting researchers. The quality of the research is a much more complex mix of factors than Clifford *et al.* allow — published floras, monographs and papers in the scientific literature are the most obvious, but accuracy in the identification of collections derives in large part from long familiarity with the collections. In turn, these identifications are the key to the literature and are thus of crucial importance to all those other disciplines that rely on taxonomists' insight and experience.

It is to be hoped that those concerned with support and management of our biological collections are not misled by the simplistic, short-sighted and ill-conceived ideas put forward by Clifford *et al.* Comprehensive, well-curated collections are essential for the production of high-quality systematic research sought by these authors.

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SIR—Clifford *et al.* raise some interesting points concerning housing collections in the natural sciences. But I will not be jettisoning any part of our herbarium.

First, plants, unlike chemical compounds, are much more complex subjects where any current description interprets only *x* out of *n* characteristics. Experience shows that the next investigator will want to see all the available material, not just an historical account.

Second, we keep well-documented specimens, as well as type(s), because the originally described material may not adequately show the range of variation in species (super-orders are too coarse a taxonomic category) and may not reflect the changing distribution of species.

Finally, reference material is needed to identify enquiries and for educational purposes, and specimens are both historically interesting and aesthetically pleasing.

At a time when many institutions in the public sector are under pressure to provide short-term solutions to cash problems, it is tempting to cast doubt on long-term scientific objectives and commitments to material culture. But with most of the world's species still to be documented, this is no time to weaken our resolve. Rather, natural scientists should focus their skills and what resources they have left in overcoming the mid-term blues. They could start with a look at collecting policies and regional needs.

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SIR—The taxonomic Brave New World outlined by Clifford *et al.* is strange indeed: natural history collections, they say, are not needed because chemists do not store the compounds they synthesize; taxonomy should be based on descriptions and type specimens and should have a rational economic basis.

But one cannot synthesize individuals as a chemist synthesizes compounds. A chemical compound and a herbarium sheet are not comparable entities, as philosophers of science have long acknowledged. To say they differ in degree, not nature, simply will not do.

Descriptions alone, even when accompanied by type specimens, are no basis for the comparative biology of the future. For one thing, descriptions are often poor representations of what is described — and, in this area, taxonomists need to progress. And taxonomists make mistakes.

By way of illustration, species limits now need extensive change in a phylogenetically critical group related to the mangosteen (*Garcinia mangostana*), found from the Philippines to New Caledonia. I know this only because I have access to collections assembled at great cost over 250 years; the descriptions of the species, even recent descriptions, are of little help, but the specimens, and some new characters, are.

Knowledge of plants in the field is important, but well-maintained herbarium collections contain a mine of information. Future monographers are likely to rely more, rather than less, on herbarium collections for an understanding of some aspects of variation patterns. These collections will more and more represent populations that have become extinct because of man's activities.

It is ironic that a photograph of Kew Herbarium should accompany the article by Clifford *et al.* Almost 100 years ago, a director of Kew, W. T. Thistleton-Dyer, suggested a similar course of action to the one they propose. He accepted variation, but thought only a single specimen of each species, representing the typical morphology of the species, was needed in the herbarium. A number of 'duplicates' were removed from the collection at Kew, and some sent to Berlin — where they were described as new species (B. Verdcourt, personal communication).

Natural history collections are a celebration of diversity, right down to the level below the species, but Clifford *et al.* rightly ask whether we need all the collections. Problems attendant on the storage of voucher specimens are real. Clifford *et al.* might also have noted that many duplicates of one genotype may be taken from a single tree, and that 20 duplicates housed in as many herbaria might be excessive, useful though they may be in evaluating variability.

One wonders if the general solution proposed by Clifford *et al.* is a justification for cutting down on herbarium activities in a particular university department; a rationalization after an all-too-common academic tragedy. Being associated both with a large herbarium and a university department, I believe that rather different kinds of research may be appropriate for the two. But the proposals made by Clifford *et al.* will help neither academic institutions nor the herbaria; taxonomy must first have a rational *scientific* basis, and to destroy the collections will not provide this.

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SIR—I was surprised that three biologists, no doubt experts in their own fields, should appear to have such naive views about the value and methodology of basic taxonomy as to suggest that our collections should be turned over to the garbage collectors. Even a superficial consideration reveals that their suggestion is completely counter to the rigorous methods entailed in scientific research, which should ensure that results are based on repeatable analyses of natural phenomena.

Clifford *et al.* say "Biological specimens may be different from [chemical specimens], but they differ only in degree not in kind". A chemical element or compound can be rigidly defined and shows variation only within certain repeatable limits, whereas a biological species is of necessity defined according to one type specimen of a species, a species being composed of many individuals showing a range of variation. It is impossible to express the nature and extent of this variation in its entirety and it is therefore necessary to sample it and to keep material for research. Chemicals do not reproduce, neither do they pass on heritable characteristics to their offspring. The destruction of herbarium material, as suggested in the Commentary, would ensure that no one could then verify or refute results which did not appear to agree with the main body of data about, say, a species.

One of the more grotesque suggestions of Clifford *et al.* is that decisions on taxonomic changes should be based solely on published descriptions. Perhaps the best way to show the fallacy of their argument is to consider one particular case.

Epling prepared a revision of the genus *Eriope* from South America in 1936. He was a careful and productive worker, but in this case his research had many defects. He recognized 21 species. If all the material had subsequently been destroyed, I would not have been able to revise the genus, finding that one species had been misinterpreted and belonged to another genus and another had been misinterpreted and belonged to a different family. Of the remaining nineteen, one was better placed in a separate genus, due to characters which Epling had failed to observe, and a further six I was able to reject as one species, as the result of careful analysis of the range in variation of the other material of these taxa available in the form of herbarium data, which Clifford *et al.* would have pulped. I also described a further three new species using material sent to me by other herbaria.

My publication on this topic, which included distribution maps to show biogeographical patterns, would have been impossible without all the herbarium material at my disposal, including not only new but also re-identified materials wrongly assigned by Epling.

As a result of my studies on *Eriope* and related genera, I was able to regroup the taxa into genera that more closely reflected their natural relationships. Nevertheless, my fieldwork has also shown me that I am not infallible, but that some opinions I held at the time of my 1976 revision were erroneous. Thank goodness the herbarium material is there to prove it.

To prepare a data-matrix for cladistic or numerical analysis, it is always necessary to re-investigate characters that may have been ignored or misinterpreted by earlier workers. This would be impossible in the taxonomic wasteland proposed by Clifford *et al.*, or perhaps these authors do not believe in such methods.

Finally, because there is at present a shortage of funding in the sciences, it is fair and proper to look at which areas need greatest priority of support. But, under these circumstances, I am suspicious of those who, through ignorance, are prepared to back the destruction of a branch of science other than their own. There is, of course, frustration that the classification and description of the approximately 200,000 species of flowering plant is still far from complete. Today, the need for this is ever more urgent, as pressure on natural environments becomes more acute. The fault, however, is not due to the lack of efficiency nor lack of desire to use modern methods, but is due to lack of resources. Science and industry rely on taxonomists, but there is less willingness to acknowledge that the quality of our expertise is inevitably related to resources put at its disposal.

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SIR—There is a gross mischief about that taxonomic research is unrelated to the curation, maintenance and expansion of herbarium collections. It has even been suggested by Clifford *et al.* that it is not necessary to maintain large collections of plant specimens in herbaria, but instead that most collections can be pulped, replacing them with computerized label data. Such a radical proposal may seem sensible and plausible to many of *Nature*'s readers, but it is based on unsound logic and a less than full appreciation of taxonomy as a science. Researchers from other fields should know why we are so upset and should appreciate how the proposals put forward by Clifford *et al.* could eventually affect them.

We have all spent time in European herbaria either because our countries did not have adequately named herbarium material, or because our herbaria lacked types and literature. It was only in these large centres that we could complete our work or find solutions to taxonomic problems raised by our colleagues.

Clifford *et al.* suggest that there is no need to collect representative specimens of plants from different localities, at different times of the year, at different stages of the life cycle, of different ecotypes, with different chemical constitution, and so on, and say that it is wasteful to keep voucher specimens for biological and chemical research. Their suggestions would lead to the destruction of the only validating evidence that the specimen(s) used in experiments was correctly identified. For instance, one of us (C.H.S.) recently collected the few surviving fragments of seeds of sophoroid legumes used by chemists to isolate new chemical compounds, only to find that some of the species were not Sophoreae and did not even belong in the family Leguminosae. There were no herbarium vouchers kept. It is probable that many anomalies in the phytochemical and cytological literature are attributable to misidentifications like these.

The preserved specimen is the only object to which data and information, current and future, applies. Preserving only the information on a specimen label would preserve only the presumed identity of the taxon. Clifford *et al.* maintain that living material can be substituted for dried collections. This is nonsense: first, it may not be possible to re-collect the species in the field, and second, comprehensive dried collections from the total distribution range give a far better idea of a species' variability than limited live collections. Further, large, living plant collections would take up expensive greenhouse space and garden facilities. So what money would have been saved?

What stability there is in the classification system is there because the material is available for restudy, fresh material is added from remaining populations, and newer techniques are applied to gather more data. This enhances the scientific value of the material that has been studied as it bears the determinavits (identification labels) of generations of revisers and links it to their and other scientists' publications.

The science of plant biogeography, which depends on the accurate identification of plants and a knowledge of where they were collected, would cease to be a viable discipline if herbarium holdings were destroyed. The demise of biogeography would make it difficult to reconstruct past climates given that many plants are sensitive trackers of climatic change. An important tool for the study of global warming would no longer be available. A similar case could be made for lichens, which are valuable in monitoring atmospheric pollution, and algae, which can monitor water pollution.

The literature-based taxonomic system proposed by Clifford *et al.*, which decides, for example, nomenclature priority on published descriptions and not type specimens, is unworkable. A specimen allows renewed interpretation and description; a published description is only one person's, sometimes prejudiced, interpretation.

The importance of making both type specimens and associated herbarium material available for study is shown by the example of the legume species *Astragalus setiferus*. If only the type specimen had been preserved we would probably not know, as we do now, that this species actually belongs to *Cornulaca* in the Chenopodiaceae. It was the critical study of more complete later collections that enabled the species to be correctly placed. There is also the problem of taxonomic uncertainty, best illustrated by the publication in the 1890s of four different classifications of the tribe Asclepiadeae (Asclepiadaceae). Any reassessment of these differing classifications will have to be based on a study of the same material.

The preservation of well-curated plant specimens in dried or liquid state in herbaria is an essential and basic scientific goal. If there is "massive duplication" that exists for some taxa in some large herbaria then there seems to be a good case to distribute that valuable material more equitably around the globe. Although some large European and US herbaria may be "creaking at the seams", many countries in the Southern Hemisphere do not have a national collection of preserved plants. In such countries, naming is often done by matching against the limited collections in universities and government departments. Despite this, the naming service is probably one of the most important taxonomic services provided to farmers, doctors, hospitals, herbicide companies, gardeners, foresters, environmentalists, conservationists, entomologists, mammalogists, ethnobotanists and ecologists in developing countries.

The real issue at stake is not that herbaria are overcrowded, or that scientific fashion seems to render herbarium taxonomy redundant or that it will be made superfluous by molecular systematics. Rather, it is that although politicians scarcely question the national and regional preservation of cultural artefacts, they fail to understand the real economic significance of the natural 'artwork' of the world, often the resources for manufactured resources. Herbaria are arks of biological diversity, which should be be esteemed for their immense aesthetic, intellectual and scientific contribution to the broader communities they serve, rather than be pulled down and their contents and expertise thrown to the winds.

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Sir—The Commentary by Clifford *et al.* is irresponsible and thoughtless. The authors say that accurate identification of taxa is of prime importance to most biological research, but they devalue the importance of herbarium specimens (except type specimens) as the basis for all botanical classification. Herbaria contain evidence of where a plant occurred and how its appearance varied over time and space, providing physical and biological parameters of the history, biology and relationships of the taxon. It is impossible to obtain these data other than by examining herbarium specimens. As the plants of the world disappear, herbaria will become a primary means of studying and understanding the principles that govern the fate of those that are left.

Type specimens of species names are essential to fix their position in the nomenclatural system. But they are not necessarily typical of the morphological variation of a species. To be an effective tool for research, a herbarium must contain a geographical and morphological range of each species.

As new methodologies revolutionize taxonomy, herbarium material is reexamined to produce up-to-date classifications. The suggestions by Clifford *et al.* that plants can be re-collected ignores the fact that in many cases the original plant populations no longer exist or have been significantly changed. Moreover, the preserved specimens obviate the need to recollect rarities, and re-collection is often costly or impracticable. With modern technologies, herbarium seeds have been used to resurrect species extinct in the wild. Many published descriptions are inadequate: a specimen is worth a thousand words. A recent study of the Australian Orchidaceae, for example, revealed that many of the names of the Australian orchids were misapplied by people who had relied on descriptions rather than referring to the original specimens.

We regard our herbaria as the most valuable asset in our broad-based research programmes. In studies of diversity, cytology, plant chemistry, agriculture, forestry and medicine, herbaria are the most cost-effective method of encompassing variation. Improvements in our knowledge depend to a large extent on the quality and selection of materials collected since the previous revision; this applies particularly to unexplored regions. Herbaria, therefore, must grow. The cost of maintaining specimens as part of a broader research programme is relatively small.

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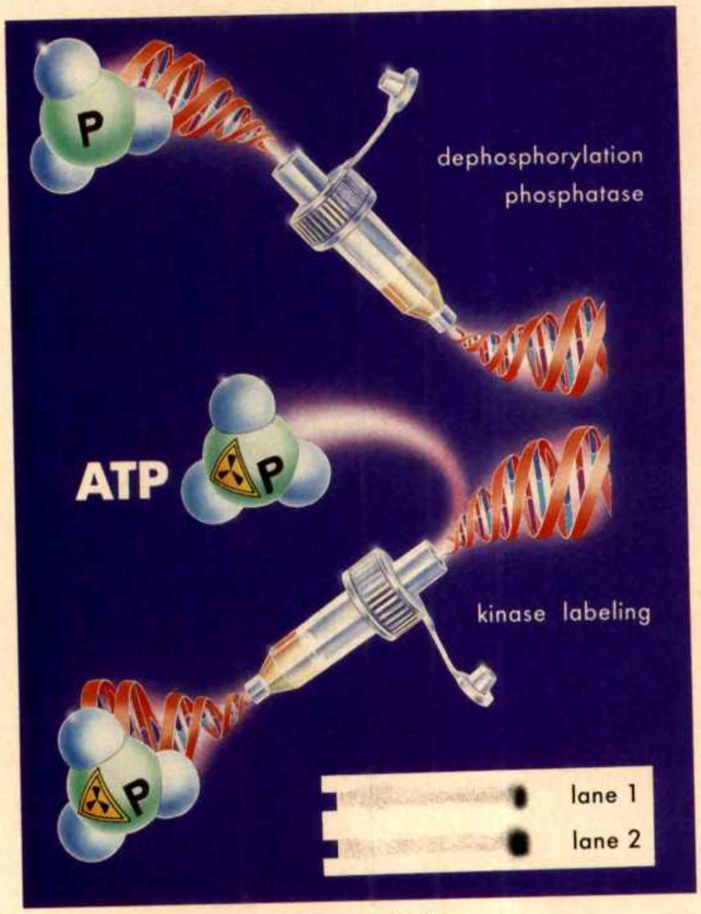

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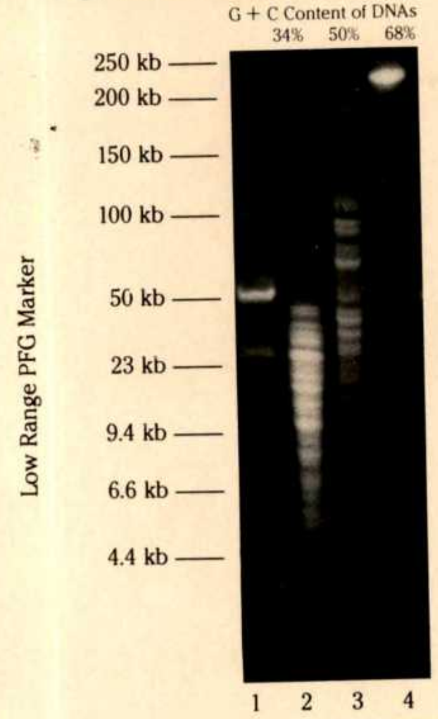

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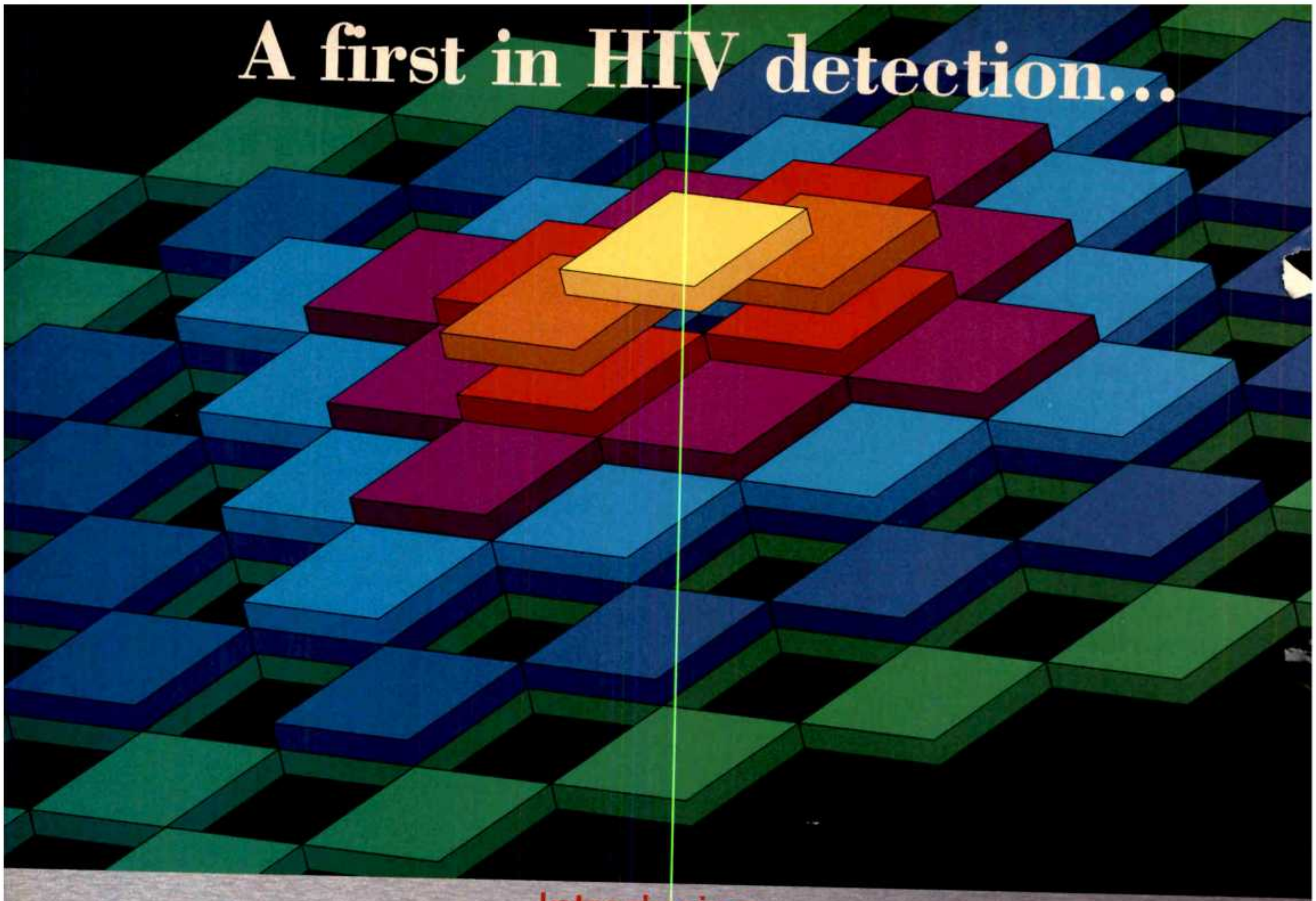
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Sandpiles as a paradigm of noise

A theory advanced in 1987 as an explanation of the ubiquitous character of noise power-spectra has been partly confirmed by sophisticated measurements of a pile of sand.

NOT often, these days, can a theorist expect an analysis to be quickly tested by experiment. In fields such as space research, people are lucky if telling observations materialize within their lifetimes. But the theory of Per Bak, Chao Tang and Kurt Wiesenfeld of the Brookhaven National Laboratory of what they call "self-organised criticality" has been successfully put to the test just over three years after publication (in *Physical Review Letters* **59**, 381; 1987).

Part of the explanation is that the challenge with which Bak and his associates faced experimentalists is at once intriguing and easily comprehended: experimentalists have enthusiastically boiled it down to the question of how the instabilities of a sandpile can be quantified. Bak *et al.* have seen their theory first denied by observation (at the beginning of last year), but now more convincingly supported. The rest of us can expect endless refinements of the sandpile measurements.

Why are they important? Bak *et al.* set out to explain the prevalence of "1/*f* noise", or the observation that the power spectrum of the noise of a variety of dynamical systems (essentially the Fourier transform of their fluctuations as a function of time) is strictly and inversely proportional to some power of the frequency (*f*) over a vast range of frequency. The noise of an electrical resistor is well-known to have this property. So does the flow of water in the Nile and the luminosity of a star.

Physically, 1/*f* noise is consistent with the expectation that smaller fluctuations will be the more common, but there are many other ways in which this might be accomplished, by an exponential decrease for example. There are important connections between 1/*f* noise and the scale-invariance of phenomena, as in fractal structures.

Why should 1/*f* behaviour be so common? Bak *et al.*'s explanation is that there is a tendency for large-scale systems to become organized into a state in which they are just on the edge of instability. The energy output from a star, for example, is determined not by the rate of energy production in its core but by that at which energy escapes by convection through the relatively cool layers just beneath the surface. So a small perturbation of the system will bring about a major change in the convection pattern. Fault-systems along which earthquakes recur are in a similar condition.

Whence sandpiles. Sand poured carefully onto a level surface will form a conical pile whose angle is determined by the properties of the sand-grains and the gravitational acceleration. But such a cone is on the edge of instability. Add only a few grains by way of perturbation, and there will be an avalanche — most probably a small avalanche, but, less frequently, a large one.

Three years ago, Bak *et al.* carried out a rudimentary numerical simulation to demonstrate the behaviour of a sandpile. The problem is worked on a square lattice, say in two dimensions. The starting-point is a random pile, which is allowed to reach stability by means of the simple rule that, if the height anywhere exceeds some arbitrary critical value, it is reduced by four units (sand-grains) while each of the four neighbouring sites is increased by one, which may mean that one or more of those sites exceeds criticality. So the process is continued until stability is attained.

Then it is possible to measure the simulated instability by notionally adding a sand-grain at random and measuring the extent of the resulting reorganization by the continued application of the rule, which is nothing but a rule specifying the working of a cellular automaton. Bak *et al.* found that the most common reorganizations were small, but the likelihood distribution of the extents of reorganization in their simulated sandpile was inversely proportional to extent over a range spanning two decades in size.

More sophisticated simulations have since been carried through; more realistic simulations of a sandpile, for example, result from rules specifying their relaxation that depend on the differences between the parameter corresponding to height at one point and at each of its neighbours. But Bak *et al.* seem also to have stimulated experimentalists to hunt for other measurable systems illustrating the principle of self-organized criticality.

For example, Babcock and Westervelt of Harvard University described earlier this year the behaviour of the cell-shaped magnetization domains in a two-dimensional film of iron-garnet doped with bismuth (*Phys. Rev. Lett.* **64**, 2168; 1990). Apparently, a magnetic field perpendicular to the film will yield a stable pattern of cells in which the magnetization is either parallel or anti-parallel to the field, but which may be dramatically disturbed by small increases of the field. Some cells grow at the expense of their neighbours, with the result that the original stable pattern is much simplified. If the size of the avalanche is the number of cells eliminated (from the arbitrary field of view of a microscope), the likelihood turns out to be inversely proportional to the size$^{2.3}$ or to the time$^{2.3}$ taken for the adjustment to be complete.

But magnetic domains are a long way from sandpiles, which may have an atavistic appeal to experimentalists. Thus Jaeger, Liu and Nagel last year announced (*Phys. Rev. Lett.* **62**, 40; 1989) that, "[a]s children we have played with sand and it is only natural to believe that we can intuit (*sic*) the processes underlying the shape and dynamics of these structures". (In passing, it may be asked why people ready to make such an interesting observation on their motives should also, with such studied inelegance, make a noun into a verb — and why their editors should let them.) The upshot of their observations, with glass beads and rough aluminium oxide particles in rotating drums using parallel-plate condensers to measure the sizes of avalanches, was that the predictions of Bak *et al.* were not verified. Rather, the authors concluded, avalanches are determined by the difference between static and (lesser) sliding friction.

Now, G.A.Held and five colleagues at the IBM research laboratory at Yorktown Heights provide evidence in the other direction. Their trick is to build conical piles of sand (again aluminium oxide particles, but sieved) on the circular pan of an electronic balance and then to add grains singly, using the balance both as a means of measuring the sizes of avalanches and, through a PC, to prevent the addition of further grains while an avalanche is still in progress. (An avalanche carries material off the pan, which is not then weighed.)

The outcome guarantees endless experiments of this kind. For smaller sandpiles, expectations are confirmed; indeed, using the interval between the addition of successive grains as the unit of time, Held *et al.* conclude that the power spectrum (the square modulus of the mass fluctuations) is inversely proportional to the f^2 for sufficiently small (high-frequency) avalanches. But, for larger piles, oscillation between the states determined by static and sliding friction matters. So now, for starters, the hunt is on to define the transition from one regime to another.

John Maddox

Righting the wrong protein

Paul M. Quinton

In the summer of 1989 the gene for the fatal hereditary disease, cystic fibrosis (CF), was identified and sequenced[1–3]. Now, barely a year later, two independent groups simultaneously report correction of CF cells transfected with the normal gene. One group (Drumm *et al.*) reports its gene complementation results in *Cell*[4]. The other presents its work on expressing and synthesizing the gene product[5] and its complementation work[6] in next week's *Nature*.

Gregory *et al.*[5] describe *in vitro* and *in vivo* synthesis of the gene product, cystic fibrosis transmembrane conductance regulator (CFTR). Like Drumm *et al.*, they surmised that previous failures to express the gene were due to toxic overexpression caused by a cryptic bacterial promoter in exon 6B of the CFTR gene. Gregory *et al.* solved the problem by incorporating the full length of an open reading frame complementary DNA of CFTR into a low-copy-number plasmid, whereas Drumm *et al.* introduced three mutations into the CFTR gene to neutralize the promoter. Gregory *et al.* went on to synthesize the CFTR gene product *in vitro* in cell-free reticulocyte lysates and *in vivo* with recombinant HeLa cells. To confirm gene expression they developed antibodies to fusion proteins containing peptides encoded by exon 10 and exon 13 of the gene, which labelled protein products of relative molecular mass about 130,000 and 140,000 (M_r 130K and 140K) from the *in vitro* and *in vivo* syntheses, respectively. The different molecular masses are accounted for by *in vivo* glycosylation. Although these results give convincing evidence of expression of the CFTR protein, the discrepancy between the apparent small size of these products compared to that predicted for the CFTR protein (which consists of 1,480 amino acids and has a putative M_r of 170K) requires clarification.

Transfection

Rich *et al.*[6] used two independent assays to demonstrate that the defect in cyclic AMP-mediated stimulus–response coupling of chloride permeability in a CF respiratory cell line was corrected in most cells when these cells were transfected with a hybrid vaccinia virus–CFTR cDNA plasmid expression system. Failures were attributed to inefficiency in transfection. Few measurements were possible because of tedious technical demands and the short life of cells after viral infection, but the results from both assays taken together offer convincing evidence that the defect was effectively complemented. Drumm *et al.* corrected the same defect in a pancreatic tumour cell from a CF patient, using a recombinant retrovirus that produced stable, viable cells and permitted a larger number of measurements using similar assays. Even though these results do not establish how CFTR affects chloride permeability, they do, of course, prove to physiologists what geneticists already knew — that the gene that was identified through 'reverse genetics' is the CF gene.

There are now at least two major routes of research to be pursued directly. First, CFTR antibodies in cells known to express CF defects must be localized cytologically. The many cell types of relatively undesignated function that comprise airway epithelia, and the relative inaccessibility of other equally complicated CF target tissues, may create confusion with respect to antibody specificity in localization studies, but the simplicity of the sweat gland could provide a standard in such assays. The sweat gland reabsorptive duct is a syncytium of cells expressing the clearest and most consistent defect in CF — impermeability to chloride ions — and the sweat gland secretory coil, which characteristically fails to respond to β-adrenergic stimulation in CF, is composed of only two very distinct secretory cells. It seems reasonable to insist that any antibody to CFTR should show binding in these two components of the gland. Antibodies meeting this criterion could then be used to identify target cells in the more complicated tissues of the airways, pancreas and other affected organs. Distinct antibodies meeting this criterion (preferably antibodies to different epitopes of CFTR) will be important in determining if other cells not currently recognized to be affected in CF also express CFTR. Antibody-localization studies seem almost certain to provide insights into the all-important question of the function of the affected protein, which is also at the heart of the second pressing route of pursuit — the biochemical properties and function of CFTR.

On the basis of homology data and modelling, Hyde *et al.*[7] have argued that CFTR is probably not an ion channel, but rather an ATPase transport protein. The course of further research, and indeed our understanding of the nature of the disease, depends on the assessment of this hypothesis. It predicts that aberrant ion-channel phenomena are secondary consequences of deranged cell metabolism and implies that the disease could be even more amenable to therapy than anticipated if the species transported by CFTR could be identified and controlled. Alternatively, if CFTR is an ion channel or a closely associated channel regulator, correction will probably require supplementing or bypassing with other components.

Now that quantities of CFTR can be produced, several crucial questions can be answered to define its properties and potential function and thereby distinguish between these two main hypotheses. Initially, we must know if the region containing the most common CF defect (a deleted phenylalanine in the 508 position of the CFTR protein) is a nucleotide-binding fold; that is, does it bind ATP or other nucleotides? If so, is it a catalytic site for ATP hydrolysis? And if it is, is it a transport ATPase or a kinase? If it is a kinase, what does it phosphorylate? And certainly, if it is an active transport protein, what does it transport?

Channel regulation

Even if CFTR does not hydrolyse ATP, nucleotides might still be involved in channel regulation. For example, there is much evidence[8] showing that the opening of a potassium-ion channel found in pancreatic β-islet cells, heart and skeletal muscle cells, and some neurons, is highly sensitive to ATP or, more probably, to the ratio of cytoplasmic levels of ATP to ADP (energy charge). Airway epithelia[9] and sweat ducts[10] are characterized by chloride conductances (permeabilities) which decrease markedly with energy deprivation. These observations could suggest that the putative nucleotide-binding fold of CFTR determines the sensitivity of an ion channel, directly or indirectly, to cell nucleotide levels, possibly in a way that protects the cell from electrolyte overloads when it is under stress or short of energy.

Whatever the actual function of CFTR, the two groups that have demonstrated expression and complementation of the CF gene have taken us to another milestone on the route from the genetic defect to effective therapy. Only a few years ago the idea of introducing normal genes into the CF lung to correct its fatal susceptibility to infection was science fiction. Now the accomplishments of these investigators seem to press the fiction inspiringly close to reality. ☐

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TRIBOLOGY

Molecular-scale lubrication

John H. Cushman

IT has been known for a few years that layers of fluid just a few molecules thick can acquire solid-like order if sandwiched between stationary, structured surfaces. According to new experiments by Israelachvili and colleagues reported in the *Journal of Chemical Physics*[1], such solid-like order may also exist when the two surfaces are in relative motion. This discovery could be said to mark the birth of molecular tribology, the study of friction and lubrication at the atomic scale.

If two surfaces are brought together to within molecular dimensions in a fluid bath, the interlayer fluid alters its dynamic and static properties. The fluid's phase diagram is changed, its thermal transport coefficients change, it becomes inhomogeneous and may become anisotropic. The properties of the fluid depend in a complex way on the initial structure of the liquid, the structure and commensurability of the surfaces, the surface–liquid potential, the separation of the surfaces, the direction of shear, the rate of shear and the history of shear. The complexity of the fluid film is born out both experimentally and computationally. All this suggests that current theories of lubrication and friction are inadequate. Just as importantly, it suggests that no conceptually correct definition or understanding of a liquid exists when a fluid is confined between two molecularly close surfaces.

In the past few years it has become routine to measure the forces perpendicular to two parallel solid surfaces separated by a molecularly thin fluid film. For film thicknesses (h) less than approximately 10 molecular diameters, the normal component of the surface force (normal stress) oscillates as a function of h with a period of approximately one molecular diameter.

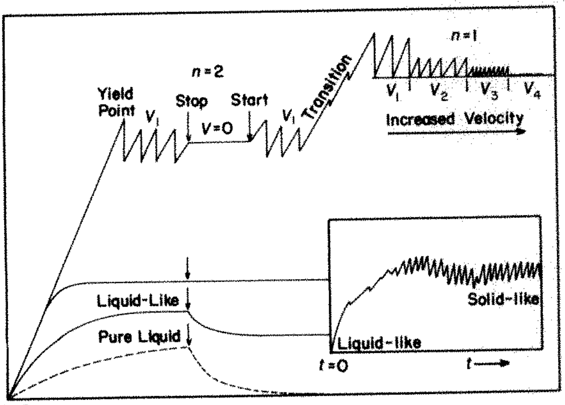

FIG. 1 Shear forces associated with different types of sliding modes as a function of elapsed time for constant load: v is the velocity of the translational stage and n is the number of fluid layers in the film. (Modified from ref. 1.)

The oscillatory character suggests that the fluid near the surfaces arranges itself in layers parallel with the surfaces and that entire layers of fluid are successively forced from the space between the surfaces as h decreases; indeed, statistical mechanics calculations confirm this notion. It was this layered structure that aroused the curiosity of tribologists. This curiosity led Israelachvili and co-workers to develop instrumentation for measuring the shear stresses a molecularly thin film exerts on parallel surfaces as they slide by one another[2].

Already the investigators have made significant progress in elucidating the strain-rate/stress behaviour of several simple fluids and mixtures of simple fluids[1]. Consider for example the experiment illustrated in Fig. 1. Two mica surfaces bathed in a fluid are forced together under a constant load that is sufficiently large to press all the fluid out of the interlayer region except for a few monolayers. One mica surface is attached to a movable stage by springs and the other surface is held fixed. The stage is set into motion parallel to the surfaces and the shear stress exerted by the fluid on the mica surfaces is recorded. The starting time which is recorded as $t = 0$ indicates when the translation stage begins moving with velocity v, but owing to the yield stress $t = 0$ does not necessarily coincide with the time the mica itself begins to move.

As indicated by the dashed line, if the interlayer separation is sufficiently large (greater than about 10 molecular diameters) the fluid behaves as if it were in the bulk phase — as shear begins the stress in the fluid builds up to a constant value and when the shearing is stopped the stress relaxes at an exponential rate to zero. As illustrated by the curve immediately above the dashed line, all of the fluids studied[1] exhibited liquid or liquid-like behaviour for film thicknesses between 5 and 10 molecular diameters. A liquid-like film is generally associated with non-spherical molecules. The main difference between liquid-like and liquid films is that in the former the stress does not relax to zero when the velocity is set to zero. This is most probably a result of the change in orientation or entanglement of the molecules.

Films of thickness less than 5 molecular diameters take on a solid-like character, as illustrated by the uppermost curve in Fig. 1. As the stage begins translating, the stress builds linearly while the surface remains in a fixed position. Eventually the yield point is reached, at which time the stress is instantly reduced. The process then repeats. The solid-like character of the film exists only up to a critical velocity, indicated by v_4 in Fig. 1, at which point the film becomes fluidic. An increase in the velocity of the stage is also commonly accompanied by a decrease in the number of fluid layers existing in the film; this is indicated by the transition in the uppermost curve in Fig. 1. As illustrated by the inset, with certain liquids the sliding starts as liquid-like and becomes progressively more solid-like with increasing elapsed time; this is generally coincident with a decrease in film thickness.

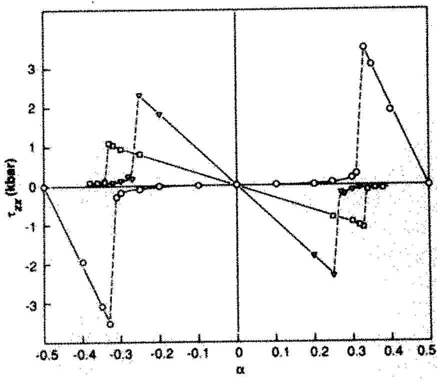

FIG. 2 Shear stress τ_{zx} as a function of registry α for surfaces separated by $h = 2.2\sigma$ (○), 3.10σ (▽), and 4.9σ (□) where σ is the diameter of the fluid (argon) atom. (From ref. 3.)

It is interesting to note that the new experimental observations for spherical molecules are qualitatively predictable and explainable from equilibrium statistical-mechanical studies of fluids in micropores[3,4] and to some degree from early continuum theories of deformation. Consider the plot (Fig. 2) of the stress exerted by an argon film sandwiched between two (100)-planes of a face-centred-cubic crystal, versus the registration of one surface with respect to the other[3]. The registration is measured by α: when $\alpha = 0$ the atoms on one surface perfectly overlie the atoms on the other; and when $\alpha = 0.5$ they are in one direction fully out of registry.

For film thicknesses less than 5 atomic diameters, the film freezes or liquefies in accordance with the registration of the two surfaces. The linear portion of the 'solid-like' curve in Fig. 1 corresponds to the linear portion of the curves in Fig. 2, as do the yield points. And the saw-toothed portions of that curve correspond to the solid/liquid/solid transitions in Fig. 2.

So the initial state of the fluid is solid and it behaves elastically (that is, its strain increases linearly with stress) until it reaches the yield point, at which time it liquefies. The surfaces then move past one another until their registration again allows the film to solidify epitaxially. The subsequent yield stresses are slightly less than the original one because the full solid structure of the original is not fully resumed: the time constant for relaxation to the solid state is too slow.

An interesting feature of Israelachvili and colleagues' solid-like curve is the transition which occurred from a film two molecules thick to one a single molecule thick, a change explicable in terms of the increased stability a film gains as it becomes thinner. This change in stability is also evident in the increased slopes and the increased values of the maxima and minima in Fig. 2. The transition is also related to the film's ability to resume a solid-like structure. If the surfaces were held at constant separation, the solvation force normal to the surface would decrease when the surfaces were out of registration. But because it is the load that is held constant in the new work, the surfaces collapse, forcing a layer of fluid out. The solid-state is then more stable and can form again.

The advent of this new experimental tool will go a long way towards developing quantitative data on complex fluid–solid interactions. In this regard, Israelachvili and colleagues have already obtained interesting data on the role humidity plays in lubrication of hydrophilic surfaces by organic liquids. Even small amounts of water (less than 0.01 per cent by weight) can drastically lower friction. The solubility of water in hydrocarbons is very low (50 –100 parts per million) and consequently most of the water in a hydrocarbon–water mixture is adsorbed to the hydrophilic mica surfaces creating a slip plane between the water and hydrocarbon. It is this slip plane that is most probably the cause for the drastic reduction in friction upon addition of water to a hydrocarbon. This information may turn out to be extremely valuable in techniques for recovering petroleum from porous media.

Although the new work gives only a glimpse into the complex behaviour of confined thin films, it provides an important starting point for many key experiments in tribology, colloid science, engineering and soil science, as well as physics and chemistry. Developments are awaited with interest. ☐

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OCEAN DRILLING PROGRAM

A new thrust at accretion

*Leg 131 shipboard scientific party**

SEDIMENT on the sea floor at a subduction zone, where an oceanic plate is driven below neighbouring continental plate, deforms and may become detached from the underlying basement rock rather than sinking into the Earth's mantle (Fig. 1). Drilling at the Nankai Trough off the coast of Japan (Fig. 2), we penetrated right through the accretionary prism of sediment made by such processes, recovering cores from the overlying accreted sediment and material from the detachment zone and below. The complex results of the tectonic forces at this active continental margin soon became apparent.

The Nankai Trough is the site of subduction of 15-million-year-old oceanic crust of the Shikoku Basin beneath the Japanese arc[1]. Our preliminary seismic survey[2] clearly showed the frontal thrust fault within the accretionary prism and a deeper seismic reflector representing the décollement surface. The décollement forms the boundary between the scraped-off sediments, which become incorporated into the accretionary prism, and the oceanic plate below, which is subducted back into the Earth's mantle.

From site 808 we obtained a continuous section through 1,290 m of sediments from the middle Miocene (15 million years ago) to the Holocene (the present) at the toe of the accretionary prism. The upper 560 m of sediments are predominantly turbidites, deposited along the trough by strong currents from the Izu-Bonin Arc – Japanese Arc collision zone to the east[3] over the past 500,000 years. The lower sediments are more characteristic of open oceans, having accumulated away from the trench in the Shikoku Basin.

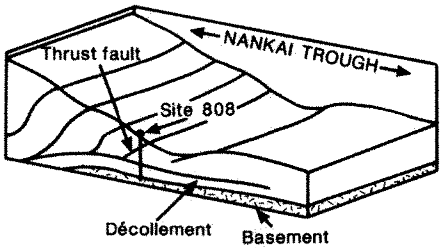

FIG. 1 Schematic of accretionary wedge at an active continental margin.

The major thrust fault 365 m below the sea floor is marked by folding of the sediments, with overturned beds just below the fault plane. There is a zone about 20 m wide, 960 m below the sea floor, containing fragmented and sheared rock. Below it, the rocks are virtually undeformed. This zone we identify as the décollement. The complete interval between the thrust and the décollement shows pervasively deformed sediments, with abundant minor faulting at depth, whereas complex shear bands predominate near the thrust fault. There is evidence that shear bands pre-date the faulting. Thus we have demonstrated that the style of sediment deformation changes with time and/or position within the accretionary prism.

The physical properties of the recovered sediments were measured at about 1-m intervals throughout the cored section. The most remarkable discovery here was the large contrast in properties occurring across the décollement. For example, there is a considerable increase in porosity below the décollement, violating the normal pattern by which porosity decreases with depth owing to compaction. This may indicate that the sediments contain interstitial pore water at higher than normal (hydrostatic) pressures.

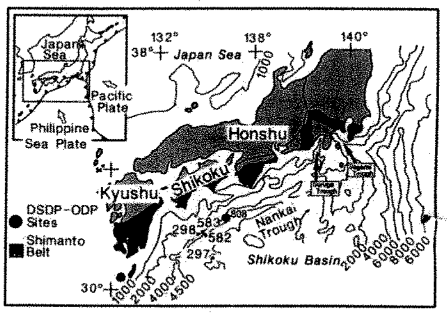

FIG. 2 Location map of site 808, Nankai Trough.

The mode of pore water expulsion from accreted sediments, whether localized along fault zones or diffused through the sediments, may affect the style of structural deformation in the prism. Our chemical analyses show that the composition of the interstitial water changes both with the changing lithology and at the two major faults. A large smooth decrease in chlorinity towards the décollement can be interpreted either as evidence of fossil fluid flow or as a result of an *in situ* reaction. But there is no direct evidence of active localized fluid flow. This contrasts with results from the Barbados accretionary prism, drilled previously[4,5], where there is strong evidence of significant recent fluid flow along the décollement and other faults.

We also made measurements of physical properties in the boreholes themselves, but only in limited sections of the holes drilled, owing to unstable hole conditions. The thermal gradient at 120 °C km^{-1} is steep, and combined with measurements of thermal conductivity indicates that the heat flux locally is 130 mW m^{-2}. An analysis of hydrocarbon gases from the hole suggests that the thermal gradient may have been even higher in the past.

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The most important single result of this leg is the quantification of the marked contrast in structure and physical properties across the décollement, which occurs despite the lithological uniformity of the rocks. There is abundant evidence of subhorizontal shortening in the accreted sediments and virtually no deformation in the underthrust sediments. Furthermore, the large offset in physical properties suggests a considerable difference in the physical conditions of stress and pore pressure above and below. Despite these differences, interstitial water geochemistry showed no chemical signature of concentrated active fluid flow. The complete absence of mineralized veins, or of any firm evidence of fluid flow, suggests that the hydrogeology of at least this section of the Nankai prism is radically different from the Barbados prism. ☐

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CELL MOTILITY

Dynamin joins the family

Peter J. Hollenbeck

IN our attempt to understand the evolution of protein function, analogy sometimes fails us. Consider two classes of proteins in particular, the mechanochemical enzymes and the GTP-binding proteins: although both classes undergo a cycle of nucleotide binding, hydrolysis and release, their relatedness has commonly been considered to end at that single shared property. After all, mechanochemical enzymes couple their nucleotide cycle to the generation of force for movement, whereas GTP-binding proteins are generally thought to couple GTP binding and hydrolysis to regulation of activity of other proteins. But work reported by Obar *et al.* on page 256 of this issue[1] now provides evidence that one mechanochemical enzyme, dynamin, belongs to a family of GTP-binding proteins, and raises the possibility that both functional and evolutionary relationships exist between proteins whose behaviour is modulated by GTP-binding, and those which generate force for movement.

Dynamin is a recently discovered mechanochemical ATPase that mediates sliding between microtubules[2]. It comprises a polypeptide of relative molecular mass 100,000 (known as D100) which is sufficient to bundle microtubules in an ATP-sensitive fashion, and an unidentified cofactor which is required for the hydrolysis of ATP and for sliding motility. Obar and colleagues obtained and sequenced a complementary DNA clone encoding the entire D100 polypeptide, and they demonstrate that it contains not only a consensus ATP-binding site, but also two additional elements indicative of GTP binding, the sequence and spacing of which are conserved among GTP-binding proteins. Moreover, there is strong sequence similarity between D100 and two related GTP-binding proteins — the Mx proteins[3] and the product of the yeast vacuolar protein sorting gene, *VPS1* (ref. 4). Obar *et al.* argue that this homology shows that dynamin and the Mx and VPS1 proteins are members of a distinct family of GTP-binding proteins. They go on to consider how a GTP-binding protein can be a mechanochemical enzyme.

Members of the superfamily of GTP-binding proteins have a common function — the development of increased affinity for a target when they bind GTP, and loss of that affinity when GTP is hydrolysed — that arose early in evolution and that has been adapted to a variety of purposes. Different families of GTP-binding proteins can be classified according to what events are regulated by their cycle of GTP binding and hydrolysis: for the G-protein[5] and *ras* protein[6] families, it is signal transduction and amplification; for the tubulins[7], polymerization and depolymerization; and for the initiation and elongation factors of protein synthesis[8], it is the unidirectional transport of aminoacyl-transfer RNAs to the ribosome. For a number of GTP-binding proteins involved in the transport of proteins into the endoplasmic reticulum[9-11], and thence to and through the Golgi towards their eventual secretion[12-14], the function of the GTP cycle is less clear. Bourne[15] suggests that these proteins are transport GTPases that drive the vectorial transport of protein-containing vesicles from station to station along the exocytic pathway. In this model, the membrane compartments of the exocytic pathway contain different marker proteins, and the transport GTPases couple the binding and hydrolysis of GTP to the loading of material at proximal markers, and unloading at distal ones.

For dynamin and its GTP-binding cousins, Obar *et al.* take the case a step further. A cycle of GTP binding and hydrolysis coupled not to regulatory interactions, but to transport, sounds rather like the force-generating cross-bridge cycle of mechanochemical enzymes. Thus, based upon both analogy of function and homology of sequence, Obar *et al.* propose that VPS1, and perhaps other secretion factors, actually serve as mechanochemical GTPases during transport, generating microtubule-based vesicle movement coupled to the binding and hydrolysis of GTP. This model differs from that of Bourne in that the information for the direction of transport could reside not only in markers on the membrane compartments, but also in the inherent polarity of microtubules and in the order in which the various membrane compartments array themselves along the microtubules. Dynamin itself would then be viewed as a member of this family specialized for microtubule–microtubule, rather than microtubule–vesicle interactions, and Obar *et al.* point out unique features of the D100 sequence that are consistent with this interpretation.

Is a system of mechanochemical GTPases consistent with our current view of intracellular transport? ATP, rather than GTP, is believed to be the physiological substrate for most mechanochemical enzymes, but it is noteworthy that all of them (including dynamin) can bind and hydrolyse GTP, and at least one — the microtubule-based motor, kinesin — can use GTP to produce movement[16]. In this context, Obar and colleagues point out that kinesin's primary sequence contains, in addition to an ATP-binding element, one of the two additional elements associated with GTP binding. This raises an interesting question. Could some mechanochemical ATPases be derived from the ancient and venerable family of GTP-binding proteins — descendants that have lost their preference for GTP, along with their ancestral function, in the ATP-rich environment of the eukaryotic cell? ☐

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MICROSCOPY

Seeing is believing

John E. Hearst

A new microscope described elsewhere in this issue by G. J. Puppels *et al.* (*Nature* **347**, 301–303; 1990) represents a milestone in the long history of cytology. The instrument can focus on a volume of less than 1 µm^3 (a volume approaching the theoretical diffraction limit of a microscope using light of wavelength 660 nm), and obtain a high-quality Raman spectrum. Because Raman-scattering spectra are sensitive to molecular vibration, such a spectrum allows one to 'see' chemical bonds, and therefore to distinguish between protein and nucleic acid.

As the technique becomes more completely developed and more rigorously applied, it could be used to explore far more subtle structural and compositional variations than are possible at the resolution of the conventional light microscope. As a new arrow in the quiver of procedures available to cytologists for determination of biological structure, confocal Raman microspectroscopy is an exceptional development.

The authors have used a combination of emerging technologies in a manner never before applied to microspectrophotometry. First, a charge-coupled device (CCD) is used as the photon detector. CCDs have been used primarily in astronomy because of their extremely high signal sensitivity achieved at low background intensities. Second, to achieve these low backgrounds, a chevron-type dielectric band-pass filter tuned to suppress the Rayleigh (or elastic) scattering of the incident 660-nm laser light with an efficiency of one part in 10^9 is used. Finally, the confocal optics allow for the tight localization of the detected inelastically scattered light to the 1 µm^3-volume element. The authors have determined the changes in the ratio of DNA to protein in salivary chromosome bands, interbands and telomeres, demonstrating the extraordinary capabilities of Raman microspectroscopy.

Genetics has had a special relationship with cytology since the 1870s, when light microscopy first revealed the existence of chromosomes. Ever since then, the quality of cytological representations of chromosomes has always outclassed molecular understanding. The cytological maps of *Drosophila* salivary chromosomes pioneered by Bridges, for example, established the relationship between genetic linkage and microscopically observable bands and distances. This work preceded the observations in 1944 by Avery and his co-workers that established DNA as the genetic carrier. In the 1960s, Joseph Gall developed the technique of *in situ* hybridization, by which many of the simple satellite DNA sequences, discovered by density gradient sedimentation and renaturation kinetics, were shown to be structurally unique to the chromosome, localizing at centromeres and telomeres. There is a major distinction between heterochromatin and euchromatin, a cytological density difference inferred to be related to biological activity. The banding patterns of salivary chromosomes and correlations between the numbers of bands and the numbers of genes in *Drosophila* make these issues even more intriguing.

The discovery of banding patterns in human chromosomes and their importance to karyotyping introduced the question again. What are the compositional and structural properties of the chromosome that create these observable cytological features, and why do they exist? Although tremendous inroads have been made at the level of DNA sequence, nucleosome structure and, to a lesser degree, chromatin coiling, we remain remarkably inept at mapping the compositional and structural properties of larger domains. Confocal Raman microspectroscopy is a most promising technique for alleviating our difficulties, for it provides *in situ* compositional and structural information without the use of invasive stains and fixatives that are common features of most cytological and microfluorometric analyses.

What remains to be seen is what else can be studied by this technique, for there are potential limitations associated with contrast and resolution. If the 1-µm^3 volume contains too complex a mixture of chemically distinct molecules, assignment of Raman lines may be impossible. On the other hand, the sensitivity of this instrument is remarkable. ☐

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ONCOGENIC VIRUSES

Of marmots and men

Don Ganem

ONE of the great unsolved problems in the biology of human cancer is the reason for the close association of hepatocellular carcinoma (HCC) with chronic infection by hepatitis B virus (HBV). This link is no small matter: over 250 million humans are chronic carriers of HBV, and in the vast areas where HBV infection is endemic, HCC ranks among the most common human tumours. On page 294 of this issue, Fourel *et al.*[1] shed new light on this connection by showing that integrated viral DNA is often linked with known oncogenes in an animal model of liver cancer.

The fact that HBV carriers are between 100 and 200 times more likely than non-carriers to contract HCC is compelling epidemiological evidence for a link between HBV and HCC[2]. Almost all HBV-related HCCs harbour integrated viral DNA, usually in multiple copies. Tumours are clonal with respect to these insertions, indicating that integration of viral DNA precedes or accompanies the transforming event.

Further evidence of a link comes from animal studies. Although HBV infection is largely restricted to humans, related viruses affect a number of other species, including woodchucks (marmots), ground squirrels and ducks. Remarkably, woodchucks infected from birth with woodchuck hepatitis virus (WHV) are almost guaranteed to develop HCC[3].

The oncogenic sword of HBV is terrible but not swift: tumours typically develop only after 20–30 years of persistent infection, and are usually accompanied by signs of hepatocyte necrosis and active inflammation (chronic active hepatitis) and fibroblastic proliferation (cirrhosis). Hepatoma induction by WHV is similar to that by HBV, except that tumours develop earlier in the course of infection and at a much higher frequency.

The molecular mechanisms underlying the connection between HBV and HCC have been elusive. The long incubation period of HCC argues against the existence of a dominant oncogene encoded by the viral genome, and most experiments designed to find one have been unsuccessful. In the absence of such a gene, most models for how HBV engenders HCC fall into two broad categories, according to whether viral DNA contributes directly or indirectly to hepatocellular proliferation. In the past year, evidence in support of both views has accumulated.

Models in which hepadnaviruses (the class of DNA virus that includes HBV) make a direct genetic contribution to the loss of growth control are influenced by studies of retroviruses (such as murine leukaemia virus, MuLV) that lack dominant oncogenes. These viruses often activate cellular proto-oncogenes by the integration of viral sequences in the flanking host DNA. But efforts to identify such oncogenes in the vicinity of integrated

HBV genomes in human HCC have generally been unsuccessful, although individual examples of HBV insertions next to genes for retinoic acid receptor[4] and cyclin A[5] have been identified. Unfortunately, these account for only a tiny fraction of human hepatomas. In this context, the findings of Fourel *et al.*[1] on WHV-related HCC present a striking contrast: of 30 hepatomas in woodchucks carrying WHV, six have insertions of WHV DNA adjacent to cellular N-*myc* sequences, and 18 of the tumours overexpress N-*myc* messenger RNA, normally undetectable in woodchuck liver. Additional woodchuck hepatomas previously reported by this group harbour WHV insertions near the related c-*myc* locus[6].

N-*myc* genes were initially discovered by virtue of their amplification in human neuroblastoma[7]: more recently, insertion of MuLV DNA adjacent to N-*myc* has been noted in MuLV-induced T-cell lymphomas, in which it is also associated with enhanced accumulation of N-*myc* RNA[8]. The activation of N-*myc* expression in woodchuck HCC is similar to that by MuLV: in both cases, insertion of viral sequences occurs in the 3′-noncoding region of the N-*myc* locus and in the same transcriptional orientation, leading to chimaeric RNA. Potent viral enhancers are thereby linked to the N-*myc* locus, as are signals for 3′ end processing. This affords both transcriptional and post-transcriptional opportunities for the upregulation of N-*myc* messenger RNA levels; which of these predominate in WHV-related HCCs remains to be determined.

Interestingly, woodchucks possess two N-*myc* loci. One seems to be the product of an ancestral reverse transcription event and most WHV insertions involve this locus. The preservation of the coding function of this gene suggests that it plays a part in normal growth and development, the nature of which remains unknown.

Besides the activation of known oncogenes, various other genetic events in flanking host DNA, such as deletions and translocations, have been associated with the integration of viral DNA[9]: these, too, could contribute directly to growth deregulation. And beyond these pathways of insertional mutagenesis are still other ways in which HBVs could influence proliferation directly. In particular, mutated or deregulated viral gene products might do so by activating important cellular functions in *trans* (this might explain those cases in which N-*myc* expression was increased in the absence of a nearby WHV insertion). The viral X-gene product is a transcriptional activator able to augment expression from many cellular and viral promoters. Although chromosomal integrations often disrupt the X coding region near its 3′ end, many such truncated products retain transactivation activity (ref. 10 and K. Matsubara, personal communication). The potential role of the X protein in hepatocyte proliferation has recently been underscored by the observation of HCC induction in two lines of transgenic mice that overexpress the viral X gene (C. Kim and G. Jay, personal communication).

The frequent rearrangements of viral DNA that accompany or follow integration can also generate novel products with the potential to alter the programme of gene expression. A remarkable example is the truncated envelope glycoprotein encoded by an HBV integrant cloned from a human HCC by Kekule *et al.*[11]. In cotransfection experiments, this protein can enhance reporter gene expression. Because the protein is both glycosylated and secreted (U. Lauer and A. Kekule, personal communication), it might act by activating transmembrane signalling pathways, though other interpretations are possible. But there is as yet no direct evidence linking the activity of this protein with cell proliferation.

A second class of explanations for the HBV–HCC connection proceeds from a different set of observations. Pathologists have long known that whereas the link between HCC and chronic HBV infection is particularly strong, hepatoma develops at a lesser but still elevated rate in other conditions characterized by long-standing hepatocellular injury, such as alcoholic cirrhosis, α1 antitrypsin deficiency and haemochromatosis. What all these conditions have in common is that hepatocyte necrosis triggers a stereotypical host response marked by chronic inflammation, fibrosis and (importantly) hepatocyte regeneration. It is presumed that this regenerative hyperplasia expands the population of cells at risk of subsequent genetic changes (mutations, chromosomal rearrangements and so on) that further deregulate cell growth. According to this view, the contribution of HBV to oncogenesis is indirect — evoking the host immune responses that lead to the destruction of infected cells and trigger this programmed response.

An important experiment[12] nicely illustrates this progression. Chisari *et al.* constructed transgenic mice that disproportionately express one of the three HBV envelope glycoproteins. This disruption of the normal stoichiometry of these proteins leads to the retention of all three in the endoplasmic reticulum in nonsecretable aggregates, the accumulation of which ultimately kills the cell. By 2–3 months of age, these mice develop liver necrosis and inflammation, with consequent reactive hyperplasia in the ensuing months. Ultimately, in transgenic lines with the greatest degrees of glycoprotein retention, HCC follows in nearly all animals. Although envelope protein dysregulation may or may not be an important cause of cellular injury in human hepatitis B (the alternative being cell lysis by virus-induced cytotoxic T cells), the potential for viral infection to set in motion this chain of events is clear.

▶

Synthetase with a difference

THE genetic code lies at the heart of molecular biology, and central to the code are the aminocyl-transfer RNA synthetases that charge tRNAs with the appropriate amino acid. The structures of the charging enzymes determine the genetic code by their combined specificity for binding particular tRNAs and amino acids. The report by S. Cusack *et al.* on page 249 of this issue describes the fourth crystal structure of an aminoacyl-tRNA synthetase (seryl tRNA synthetase, at left above). Remarkably this structure bears no resemblance to the three aminoacyl-tRNA synthetase structures previously determined (for example that of tyrosyl tRNA synthetase, right), and thus suggests a deep division in the genetic code.

David Blow

The three aminoacyl-tRNA structures previously known all share a common structural motif — the dinucleotide-binding fold that interacts with the ATP that drives the charging reaction. It had been speculated that all the charging enzymes would have this fold and thus share a common origin in the distant evolutionary past. The new results with the sequence analyses of G. Eriani *et al.* in last week's issue (*Nature* 347, 203–206; 1990), show that this is not so, and that aminoacyl-tRNA synthetases fall into at least two classes with no sign of any evolutionary relationship. The significance of this division is an outstanding puzzle in the evolution of the genetic code. G. N.

Can these divergent views of the contribution of HBV to oncogenesis be reconciled? There is no reason why both direct and indirect mechanisms cannot operate *in vivo*. The fact that liver injury of any cause invariably provokes hepatocyte proliferation means that even in the absence of a specific genetic contribution from the viral genome, there is likely to be a background of transforming events not directly linked to viral DNA. For proponents of direct models, the challenge now is to define to what extent subsets of human HCC exist in which integrated HBV sequences act to drive proliferation over and above this background, and to clarify the mechanisms by which this occurs. Clues to such cases might emerge from consideration of the differences between WHV- and HBV-associated oncogenesis, chief among which is the earlier onset of hepatoma during chronic WHV infection. Perhaps the subset of human HCCs that develop earlier than usual in the carrier state would be enriched for such integrants. The finding of frequent oncogene activation in woodchuck HCC will surely invigorate the search for comparable events in human hepatomas. ☐

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GEOPHYSICS

Enhancing mantle conductivity

L. M. Hirsch

THE cause of a layer of high electrical conductivity in the Earth's upper mantle[1] at depths in the region of 40–180 km has long intrigued and puzzled geophysicists. Both above and below the layer, conductivity profiles can be reconciled with laboratory conductivities of mantle minerals under controlled conditions[2,3]. Conversely, conductivities in the high-conductivity layer are about 1–2 orders of magnitude greater than would be reasonably expected from solid-state conduction in the volumetrically most important minerals. Small amounts of good conductors such as partial melt[4], water[5] or carbon[2] are often invoked to explain this anomaly. The common approach is to treat the layer as a mixed medium containing insulating silicate crystals mixed with an interconnected fluid or grain-boundary phase of high conductivity. On page 272 of this issue[6], Karato presents a new possibility: hydrogen dissolved in the olivine lattice may substantially increase the electrical conductivity of the host crystal. Thus, enhanced conduction outside mineral grains would not be necessary.

Karato bases his idea on diffusivity measurements of hydrogen[7], assuming that the hydrogen contributes as charged ions, presumably protons, to electrical conduction in olivine. Because hydrogen is mobile in the [100] crystallographic direction of olivine, only a modest amount of hydrogen — H/Si content between about 200–2,000 parts per million — would be sufficient to account for the high-conductivity layer. Although direct evidence of such hydrogen concentrations within olivines and their fluid inclusions from most mantle-derived xenoliths is limited[8], this circumstance may simply be caused by hydrogen loss during ascent because of its rapid diffusivity in olivine[7].

Many mechanical and petrological stability problems are avoided with Karato's hydrogen hypothesis because the hydrogen is intracrystalline and the amount of hydrogen required to enhance conductivity is below that which would cause extensive partial melting[6]. In contrast, with the water or melt hypotheses, one is left with the problem of explaining how the mechanical stability of an interconnected fluid phase can be maintained over long distances in the upper mantle for long geological times[4]. And the carbon explanation is constrained by the temperature and pressure for diamond stability, the grain-boundary diffusivity of carbon, and oxidation state of the upper mantle[2].

To verify Karato's hypothesis, several issues need to be resolved. First, it must be experimentally demonstrated that hydrogen exists as a charged defect in olivine at high temperatures and pressures. Second, we need estimates of the hydrogen content of the upper mantle and the relative partitioning of hydrogen among co-existing mantle minerals. Third, we require a chemical rationale that explains why enhanced conductivity by hydrogen does not persist beyond the high conductivity layer at both shallower and deeper depths. Finally, we need to evaluate what influence the considerable anisotropy of hydrogen diffusivity in olivine, spanning two orders of magnitude[7], may have on the amount of hydrogen required for enhanced conduction. For isotropic mixtures, this anisotropy would increase by a factor of only about 3 Karato's values for the hydrogen content. But significant texture of olivine in the region of the high-conductivity layer could produce a more profound effect on hydrogen-enhanced electrical conductivity.

The cause of the high-conductivity layer is a critical question in geophysics. This layer is located in a region of the upper mantle that has several remarkable features that are intimately connected with the thermal and rheological state of the Earth's mantle and crust. Beneath the rigid lithospheric plates (70 km thick on average) there is the asthenosphere, a more plastic layer that permits vertical isostatic adjustments of land masses and lateral shear to occur. This shear is driven by convective motions in the mantle and has a direct bearing on the motion of the plates and surface deformations. Another feature is the low-velocity zone — an effective decrease in seismic-wave velocity that starts within a depth range of about 50 to 220 km. Many geophysicists argue that partial melting gives a comprehensive explanation for the low-velocity zone, high-conductivity layer and the weakness of the asthenosphere. The mechanisms responsible for each of these features, however, could differ: both the high-conductivity layer and the low-velocity zone are often absent beneath continental shields whereas isostatic adjustments from the last ice age, such as in the Canadian and Baltic shields, indicate the presence of the asthenosphere. Yet, although there is no necessary connection between high conductivity and asthenospheric plasticity, hydrogen concentrations of the order 100–3,000 p.p.m., such as invoked by Karato, may also weaken the upper mantle by a factor of 1.5–3 (refs 9,10).

Of course, high electrical conductivity can have many causes. We may find that high conductivity layers in different localities in fact have different origins. ☐

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SUPERFLUID HELIUM

Rotons put physics in a whirl

P. V. E. McClintock

WHAT is a roton? The existence of rotons, the particle-like excitations that carry much of the thermal energy of superfluid helium-4, has been thoroughly substantiated through numerous experiments and much is known about their properties. Yet the physical nature of the roton remains almost as obscure as when its existence was first proposed by Landau[1] half a century ago. But a little progress is now being made towards a resolution of this question and it formed one of the main topics at a recent conference* on quantum fluids.

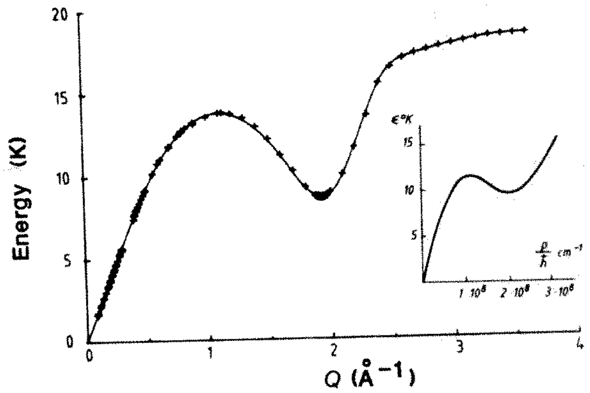

FIG. 1 The experimental dispersion curve for the excitations in superfluid helium-4 under its saturated vapour pressure in the low-temperature limit. Their energy (divided by Boltzmann's constant *k*) is plotted as ordinate against their momenta (divided by Planck's constant ħ) as abscissa. The excitations near the origin are phonons; those near the local minimum are known as maxons; and those near the local minimum are called rotons. (From ref. 5 based on data from ref. 6. Inset is Landau's prediction.)

Rotons are just one species in the bestiary of collective excitations found in condensed matter. Like phonons, the vibrational excitations of a crystal lattice, or photons, the excitations of the electromagnetic field, these quanta of superfluid helium-4 behave like particles insofar as they carry measurable momentum and energy. Indeed, their best characterized aspect is undoubtedly their dispersion relation, connecting these quantities.

This can be measured by scattering beams of neutrons off the superfluid helium: any change in the neutron's momentum and energy must be attributable to the creation or destruction of one or more excitations. The compiled data (Fig. 1) make a curve remarkably similar to Landau's inspired guess (inset on Fig. 1) which he based on early measurements of superfluid helium's specific heat capacity. Rotons are the excitations that lie close to the local minimum in the curve. The other excitations portrayed, very different in character, are phonons in the linear region at low energy, and 'maxons' close to the local maximum.

In the roton region, the dispersion curve takes on a parabolic form, just as one gets for a free particle moving in a vacuum. But there is an important difference: the minimum of the curve comes at a finite momentum rather than at zero momentum. A roton at the minimum consequently has the curious property of remaining stationary in the liquid (the speed, or 'group' velocity, of an excitation being determined by the gradient of a tangent to the dispersion curve), despite its large momentum. Rotons just to the left of the minimum which, even more astonishingly, move backwards in the opposite direction to their momenta, are known as R^- rotons; those on the high-momentum side are called R^+ rotons.

It has long been held that a roton is probably surrounded by a dipolar velocity field so that the superfluid streams around it in a pattern similar to the distribution of magnetic field round a small bar magnet. R.J. Donnelly (University of Oregon) has now used this idea to calculate how rotons are scattered by quantized vortices (the quanta of rotation) in superfluid helium-4 (Fig. 2). It can be seen that rotons colliding with the left of a vortex with clockwise circulation are only marginally deflected; whereas those to the right suffer an almost total reversal of direction, undergoing a species change from R^+ to R^- rotons. This approach yields values of the so-called mutual friction parameters that are in good agreement with those values derived from thermal transport measurements.

Although the dipolar velocity field provides a good picture of the roton when viewed from afar, it gives little in the way of clues as to the nature of the enigmatic entity at its centre. G.A. Williams (University of California at Los Angeles) reminded the meeting that Feynman's intuitively appealing picture[2] of the roton as a microscope vortex ring would yield the right kind of flow field although, according to Roberts and Jones' calculations[3] of a few years ago, it is apparently not correct in its original form. More experimental evidence is needed.

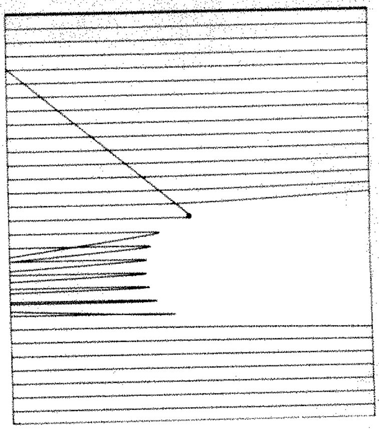

FIG. 2 Calculated trajectories of rotons incident upon a vortex line (indicated by the dot) with clockwise circulation. (From ref. 7.)

One of the few ways of obtaining information about the roton core, borrowing a technique from particle physics, is to study the interactions of colliding beams of rotons. Making a pair of crossed-beams of rotons by pulse heating a tank of superfluid helium-4 with two thin-film contacts, and detecting them via the free atoms they eject into the vacuum above the superfluid's surface, A.F.G. Wyatt (University of Exeter) and colleagues discovered that the scattering cross-section is remarkably momentum dependent (Fig. 3).

The origin of this intriguing effect is still controversial, Donnelly and I. Iwamoto (University of Tokyo) offering competing explanations. But given that the cross-section for beams of featureless hard spheres is constant – the experiments must be telling us something quite fundamental about the nature of the roton.

The new information coming from recent neutron scattering experiments is complementary to such studies. It amounts to much more than just the mapping out of the low-temperature dispersion curve of Fig. 1, which is merely the trace of the maxima of scattering peaks. The intrinsic width and structure of a particular scattering peak is replete with detailed information. The temperature

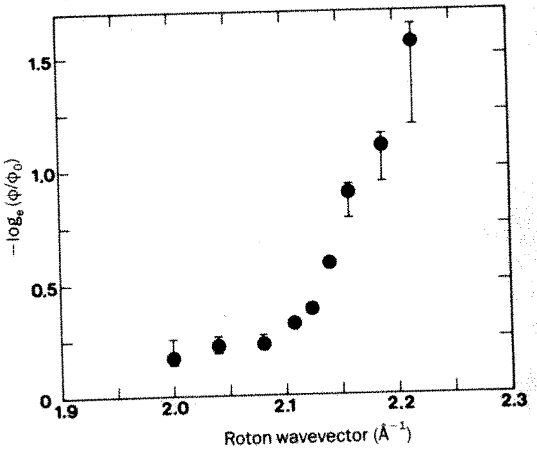

FIG. 3 The attenuation (proportional to the roton–roton cross-section) of two beams of rotons colliding at right angles, measured as a function of the momenta of rotons in one of the beams. (From ref. 8.)

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* NATO Advanced Research Workshop *Excitations in 2D and 3D Quantum Fluids*, Exeter, 10–15 August 1990.

dependence of the scattering intensity is of particular interest in that, for certain parameter ranges (such as the maxon and roton regions) it consists of two principal features: a sharp peak superimposed upon a broader background (Fig. 4). It is evident that the sharp peak disappears above the transition temperature, T_λ, at which the superfluid turns normal.

Considerable debate centred on the physical significance of the relative intensity of the two peaks in Fig. 4. A widely accepted perception, clearly articulated by A. Griffin (University of Toronto), is that the phonons on one hand, and the rotons and maxons on the other, are inherently different kinds of excitation — quite to the contrary of what one might assume from the continuous character of the dispersion curve in Fig. 1. Phonons, being density fluctuations of the whole fluid, are detected by neutron scattering both above and below T_λ. Rotons and maxons, on the other hand, are more localized 'single-particle' excitations of some (unspecified) kind, and are probably characterized by an entirely distinct dispersion curve above T_λ at which temperature they cannot, unfortunately, be detected by neutron scattering. Below T_λ, the existence of a small fraction n_0 of the helium atoms occupying the zero-momentum 'Bose-condensed' state has profound theoretical consequences. In particular, it causes the phonon and roton/maxon branches of the dispersion curve to combine, through a quantum-mechanical process known as hybridization, thereby forming the single dispersion curve observed in the experiments. Correspondingly, the maxon/roton excitations then become detectable by neutron scattering (the sharp peak evident in Fig. 4) with a weight that is proportional to the fraction n_0.

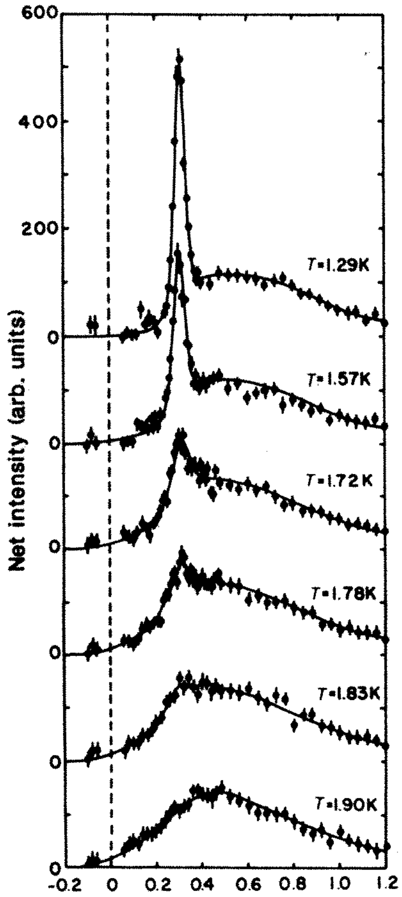

FIG. 4 The neutron scattering intensity from superfluid helium at the maxon, plotted as a function of energy (divided by Planck's constant $\hbar$) for several temperatures. For the ambient pressure of 20 bar, the superfluid transition temperature T_λ = 1.93 K. It is evident that the sharp peak disappears at T_λ. (From ref. 9.)

This argument depends, of course, on the existence of a non-zero condensate fraction (which should be carefully distinguished from the superfluid fraction, representing the proportion of the fluid which displays superfluid properties and which approaches 100 per cent below 1 K). Fortunately, n_0 itself can also be measured by inelastic neutron scattering, albeit in collisions with a much higher transfer of momentum. P. E. Sokol (Pennsylvania State University) was thereby able to derive a value of $n_0 \sim 0{\cdot}10$ as $T \rightarrow 0$, agreeing within experimental error with earlier neutron results by Svensson and co-workers[4] based on a rather different approach. This is of course very much a finite value and it is also consistent with recent theoretical predictions.

Given that rotons crop up in all facets of superfluid helium-4 research — light-scattering studies, diffusion of helium-3 through the superfluid, and the crystallization of solid helium-4, for example, all discussed at the meeting — it is remarkable that so little is known of their physical character. But then, after 50 years of study, superfluid helium-4 remains in many ways much more mysterious than its younger relatives superfluid helium-3 and superconductivity. The prospect held out at the meeting is that we should soon come to grips with what makes it so much more complex. □

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DAEDALUS

Deep digestion

MOST land animals use the nitrogen in their food proteins very inefficiently. They excrete it as urea or uric acid, making no attempt to burn these waste products usefully. Yet urea and uric acid are quite feasible fuels. As manufacturers of high explosives know, an oxidative decomposition which releases free nitrogen gas also releases a lot of energy. Why don't animals exploit this energy?

The reason, says Daedalus, is that nitrogen is almost insoluble in water. Carbon dioxide, our main gaseous waste product, is soluble enough to be transported round the body in solution. But a creature that generated nitrogen internally would find itself in trouble. It would swell up with bubbles of undissolved gas, and might even burst.

Divers risk a similar fate. Under the high pressure of the depths, the nitrogen in the air they breathe dissolves in their body fluids. When they come back to normal pressures it comes out again, and may form bubbles in the blood or synovial fluid. This is the painful and dangerous diving syndrome, 'the bends'.

So, says Daedalus, the ultimate metabolic efficiency should be shown by deep-sea creatures, living under pressures high enough for nitrogen to be easily soluble in water. Freed from solubility problems, they could metabolize proteins and other nitrogenous foods completely to nitrogen, perhaps feasting on the steady fall of nitrogenous wastes from the low-pressure creatures far above them.

DREADCO's biologists are now studying deep-sea lantern-fish, worms and sponges in special pressurized aquaria and culture vessels, looking for novel enzymes that metabolize urea and other nitrogenous wastes. If they find them, they will look for traces of them in land creatures too. A small amount of nitrogen metabolism, not enough to cause the bends but giving a little added efficiency while slightly shrinking the excretion load, could well have evolved in many species. Selective breeding, or even genetic engineering, could develop it further.

At first Daedalus dreamt of breeding a special non-excreting urban dog, which metabolized its food all the way down to nitrogen gas; but he soon realized that at atmospheric pressure it would rapidly succumb to the bends. He then recalled his previous scheme for a high pressure hen house or turkey coop, to revive the flight instincts of these sedate birds. So he now plans to develop a breed of non-excreting high-efficiency chickens, turning nearly all their food into meat and eggs with minimum waste and mess. Their special coop will be pressurized with oxygen and argon, and tended by farm labourers in diving gear.

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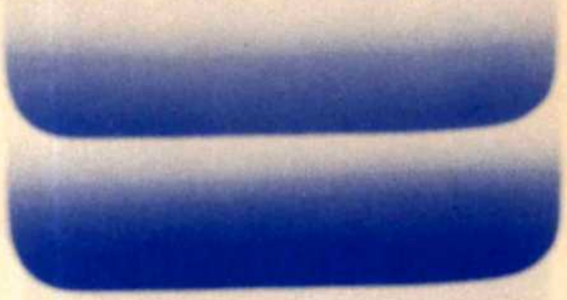

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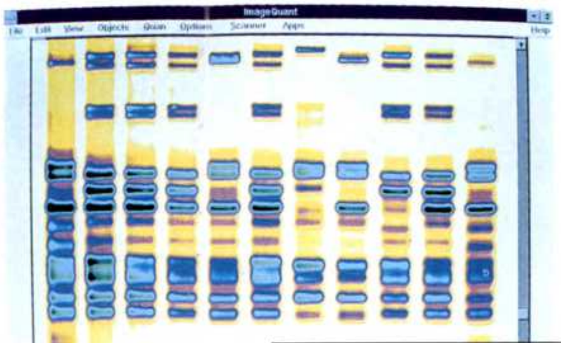

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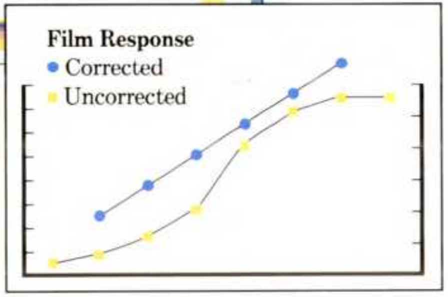

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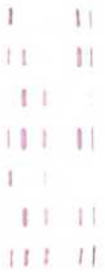

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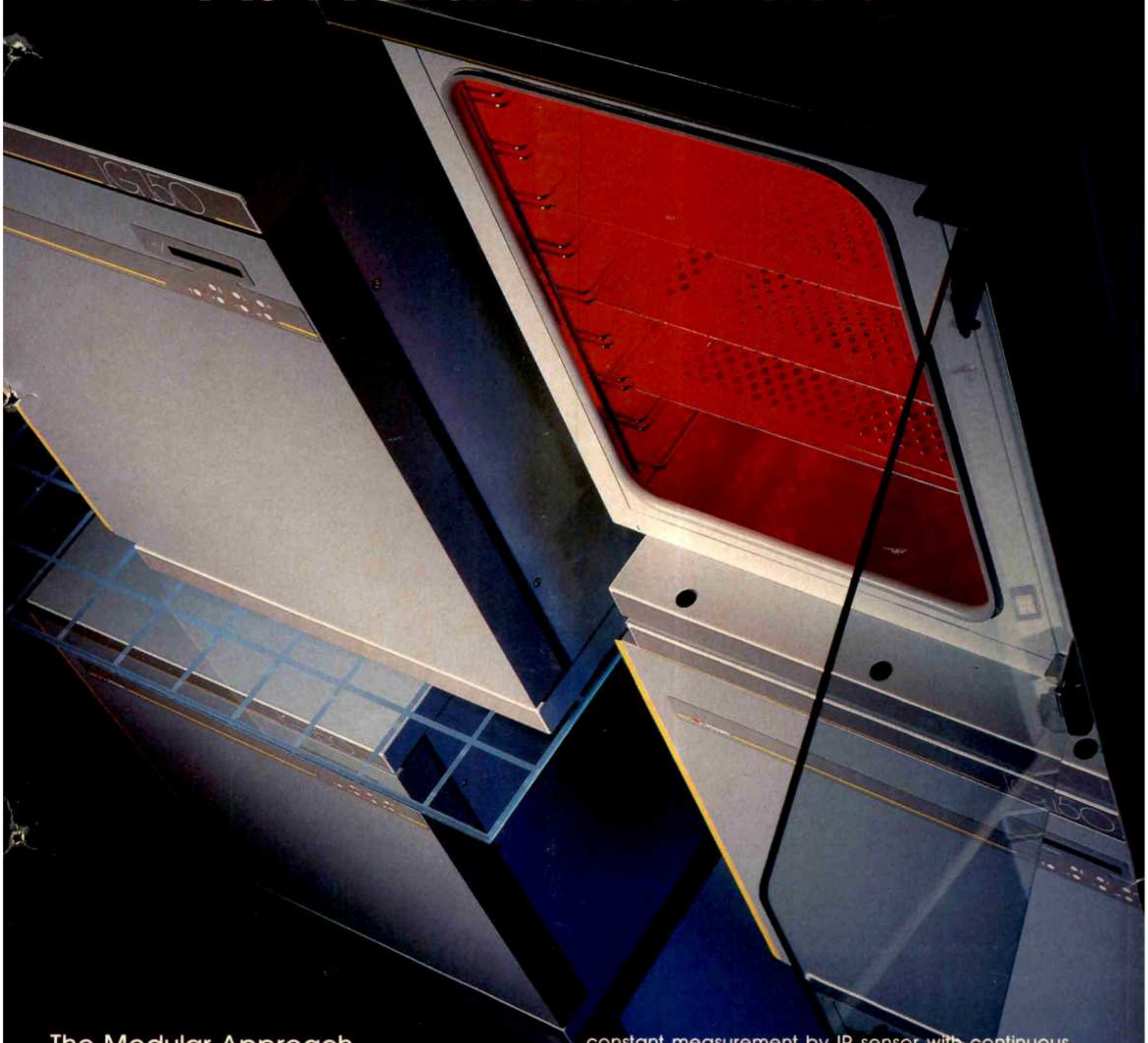

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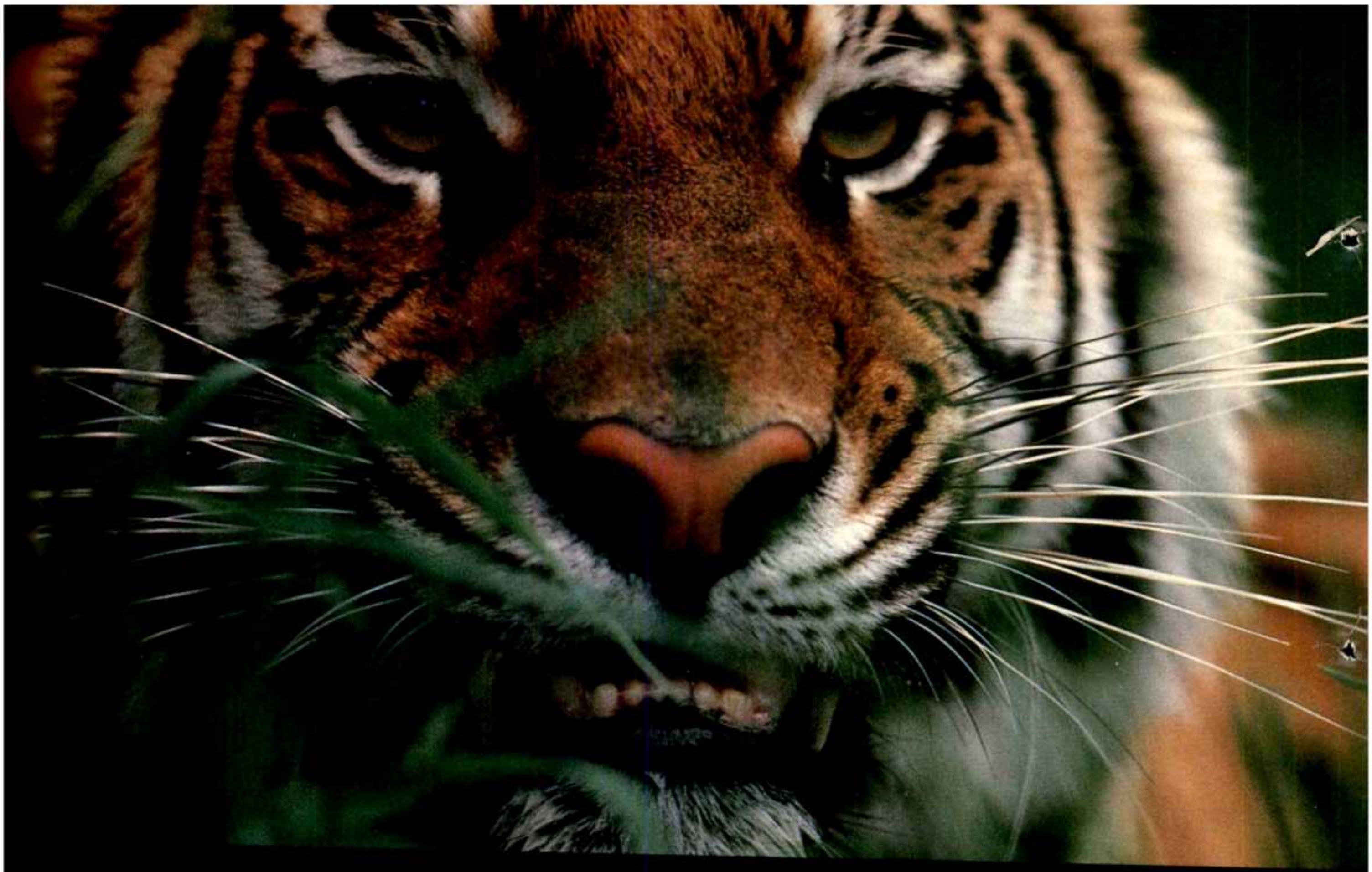

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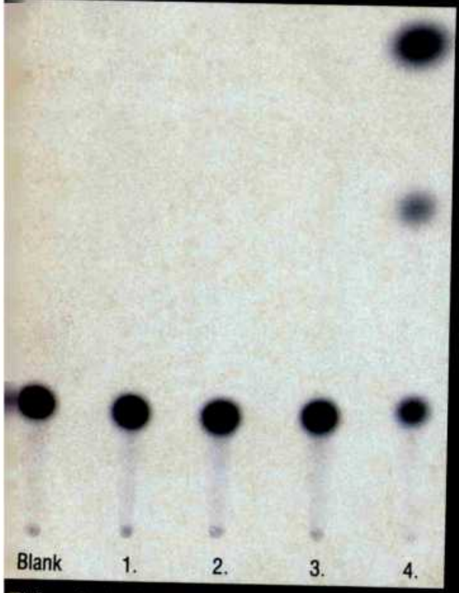

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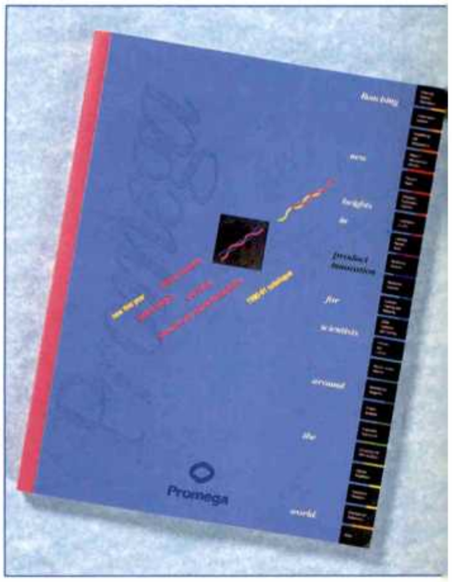

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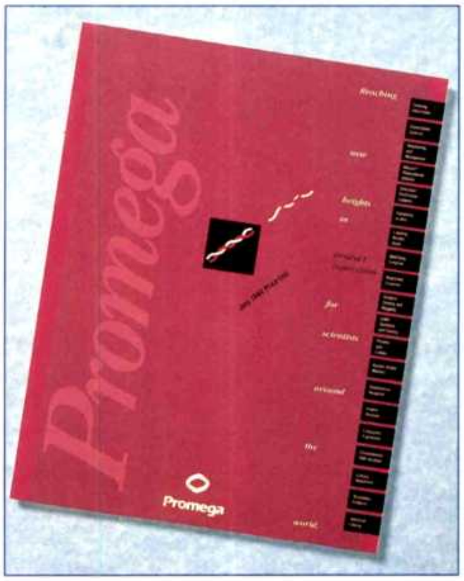

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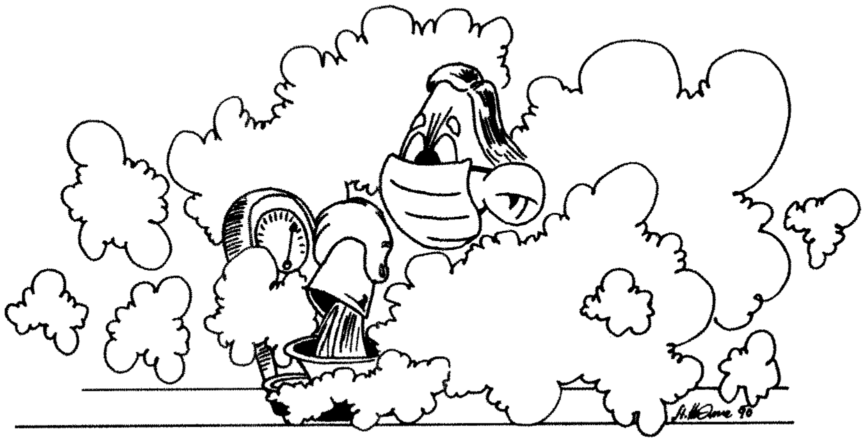

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Global warming and wheat

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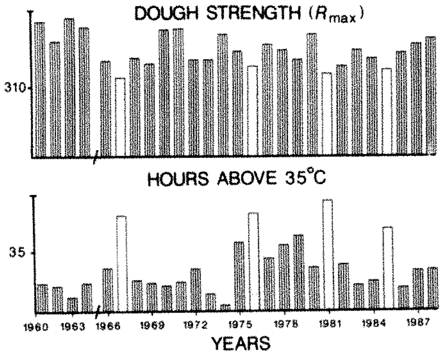
High growth temperatures are important factors in wheat-growing countries such as Australia, where temperatures during grain-filling often rise well above the moderate (say 30 °C) temperatures traditional for cereal growing. Such temperature fluctuations (say more than 35 °C for only a day or two) could account for previously unexplained changes in crop quality, according to our research.

Our survey[1] of Australian prime hard wheat quality during the past decade (see figure) indicates that the seasons when dough strength was at its lowest (irrespective of changing variety mix) occurred in those years with the greatest number of hours over 35 °C during the grain-filling period. Retrospective study of wheat-variety trials verifies this trend: 'hot' seasons were associated with weaker dough properties (and thus poorer breadmaking quality) for all but one variety. Possibly some such genotypes will provide genetic sources of tolerance to heat.



Weaker dough strength for Australian prime hard wheat coincides with high-temperature episodes. During the past decades, those seasons when dough strength (R_{max}) was least were the ones with the hottest ripening periods (hours above 35 °C at Narrabri from 1 October to 12 December).

We have performed some experiments to explain (and possibly counteract) the dough-weakening effect of high growth temperatures. Wheat seedlings synthesize heat-shock proteins in response to an hour's exposure to 40 °C of heat. Some of these proteins are similar to the range produced in other organisms, but we detected one characteristic 14-amino-acid peptide in coleoptiles and roots.

Twelve of these residues are identical to the 12 amino-terminal residues of α/β gliadins, previously found exclusively in the endosperm of wheat grain. Furthermore, the location of the gene encoding this heat-induced peptide (on the short arm of chromosome 6D) is the same as that encoding α/β-gliadin[2].

Examination of published nucleotide sequences of cereal-storage proteins reveal the presence of five heat-shock elements (the sequence responsible for switching on heat-shock proteins at high temperature) only in the leader sequence of α/β-gliadin. Increased synthesis of gliadins (part of the wheat–gluten complex) would account for the dough-weakening effect of high temperatures. That gliadin synthesis does increase under such circumstances has been demonstrated with heads in liquid culture. Furthermore, grain exposed to high temperatures in the glasshouse and the field has more gliadin and a weaker dough property than normal.

If greater temperature fluctuations occur, as predicted by the greenhouse model, periods of high temperatures during the grain-filling period of temperate cereals are likely to increase in both frequency and severity. Our results may suggest a method for the genetic modification of temperate cereals to meet the challenge of increasing global temperatures.

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Radioactive dust

SIR—In his recent Scientific Correspondence[1], Taisei Nomura said that "some studies at Sellafield have revealed an unusually high contamination of workers' homes with radioactive materials". The reference quoted by Nomura was an editorial by Beral[2] who used essentially the same phrase indicated above but referenced our work[3]. In that study, we identified one radionuclide of possible workplace origin and commented on its minor dosimetric significance.

Our study, conducted between 1984 and 1986, was designed to identify any enhanced levels of radioactivity in domestic dust and to assess its radiological significance. It encompassed 14 communities in west Cumbria in the north of England; we sampled about 170 houses including 25 each in Seascale and Ravenglass. We found various radionuclides of Sellafield origin; alpha-emitting nuclides contributed most to committed effective dose equivalent (CEDE).

We concluded that estimated CEDE values were small in relation to natural background radiation and represented a very low level of risk. In two houses in Seascale, we found elevated concentrations of the gamma-emitting nuclide ^{60}Co, probably of workplace origin. But the dosimetric consequences of these levels was small compared with that referred to above from other radionuclides; for the ingestion pathway approximately 7% of the total for an adult and 1% for a 1-year-old child. There is evidence, therefore, that, over the period of our study, contamination of workers' homes was of minor dosimetric significance compared with the general indoor exposure of Sellafield origin and that this in turn constituted a very low level of risk.

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Ozone depletion

SIR—In a recent NASA/WMO report[1], it was suggested that a 2–3% decrease in total ozone occurred in the mid-latitudes of the Northern Hemisphere during 1969–86. Depletions of up to 6% may occur in both hemispheres in future depending on man's ability to limit emissions of certain halogen-containing trace gases. But the development of the ozone hole in the stratosphere over Antarctica during winter[2,3] has more serious short-term implications. This ozone depletion, of up to 75%, could be due to reactions involving chlorofluorocarbons[4].

Atkinson and Easson reported[5] record low total ozone concentrations over southern Australia during December 1987. In particular, they found a reduction of 25 Dobson units (DU) in Perth on 11 December and a 35 DU decrease in Melbourne between 11 and 13 December, similar with effects in Hobart and Lauder, New Zealand. These low values were thought to be due to the transport of ozone-depleted air from the Antarctic[6].

The Australian Radiation Laboratory has broadband solar ultraviolet radiation detectors at 14 locations throughout the country and at the three permanently manned Australian stations in Antarctica. But for December 1987 only Melbourne data were available, comprising spectral measurements on cloud-free days and continuous broadband measurements. We have analsyed these data to gauge the impact the ozone depletion and the subsequent increase in solar ultraviolet radiation may have had on exposed people.

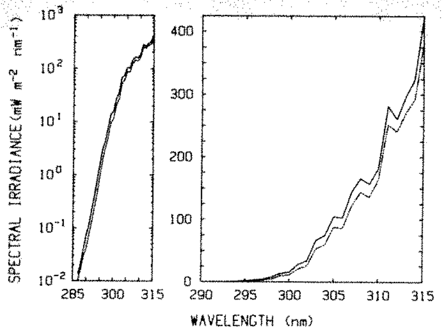

FIG. 1 Solar ultraviolet B spectral power distribution for 10 and 14 December 1987. (Broken and solid lines, respectively.) Measurements for Melbourne under cloud-free conditions.

We used a microcomputer-controlled spectroradiometer incorporating a double-grating monochromator[7] to measure global ultraviolet radiation incident on a horizontal plane. The broadband measurement system comprises four radiometers (solar radiation, total ultraviolet, ultraviolet B and actinic B) and acquires data continuously. Figure 1 shows the solar ultraviolet B spectral power distributions obtained at solar noon on 10 and 14 December — that is, before and after the event described by Atkinson *et al.*[6]. The percentage increase in the spectral irradiance for 14 December over that for 10 December as a result of the ozone decrease was 50% (at approximately 297 nm), 25% (at 303 nm) and effectively 0% (at 325 nm). Such variation results from the rapid decrease in the ozone absorption coefficient across this wavelength region.

Significantly, ozone levels dropped on 10 December and remained low until 19 January 1988. Figure 2 shows the solar noon ultraviolet B irradiances, obtained by integrating the spectral irradiances over 285–315 nm. The broadband ultraviolet B detector gave a similar profile and as expected, there is a negative correlation between these data and the midday ozone column.

Between 10 and 14 December, the 10.5% decrease in ozone concentration resulted in an 11.9% increase in ultraviolet B irradiance and about a 21% increase in the effective irradiance[8]. Hence, at this latitude, the percentage increase in effective ultraviolet B irradiance for a 1% decrease in ozone, is about two.

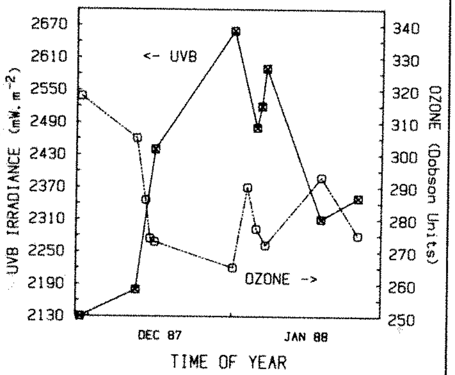

FIG. 2 Comparison of solar ultraviolet B irradiance and total ozone for several days during December 1987 and January 1988. Ozone data provided by R. Atkinson of the Australian Bureau of Meteorology.

There is concern over whether this episode of ozone depletion will have harmful effects such as an increase in the already high rates of skin cancer. But because of increased cloud cover, total ambient ultraviolet B radiation for December 1987/January 1988 was no greater than that of December 1988/January 1989, even though higher irradiances were detected on the cloud-free days of the 1987/1988 period.

We conclude that ozone depletion occurring in Antarctica is already having an effect on populated regions of the Southern Hemisphere as a result of the transport of air after the spring break-up of the Antarctic vortex. The effect may be greater in future if an ozone depletion period were to coincide with an extended cloud-free period.

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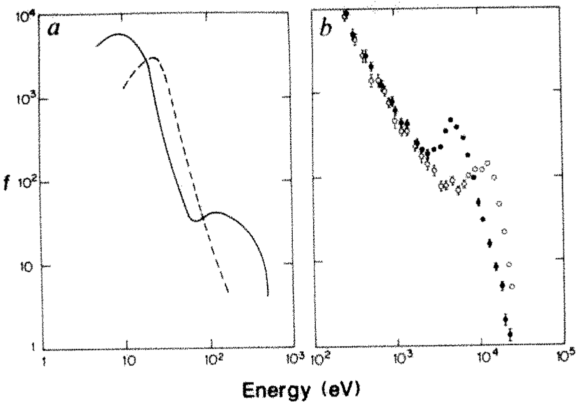
Martian electron spectra explained

SIR—Last year it was reported by N. M. S. *et al.*[1] that when the Soviet spacecraft Phobos approached Mars on 12 February 1989 and entered the magnetosheath of heated solar wind behind the bow shock upstream from the planet, the energy distribution of electrons was generally characterized by two distinct peaks. We have been studying the energization of electrons in the aurora and other space plasmas, and would like to suggest an explanation for this observation, based on an energy-selective acceleration of solar-wind electrons by electrostatic waves at the martian bow shock. If our explanation is correct, this would add further weight to the growing evidence for the universality of electrostatic-wave acceleration of electrons in space plasmas.

Figure 4 of ref. 1 shows an electron spectrum (count rate as a function of energy) observed in the martian magnetosheath at 18:36 UT. This spectrum exhibits two peaks. In our figure (*a*) we translate the spectrum into a distribution of relative velocity-space densities, *f*, by dividing count rates by the square of kinetic energy. In a dynamical system (not the case here), *f* will remain unchanged, even though the energy at which a given value occurs may vary (Liouville's theorem). The solar-wind distribution, observed 31 min earlier at 18:05 UT, and from which the magnetosheath distribution is derived, is added for comparison. Acceleration at the bow shock has introduced the new features of a minimum in *f* at 70 eV, a peak at 115 eV and a high-energy tail, such major distortions signifying a non-dynamical process in operation. The process may also have caused the reduction in *f*, or equivalently the reduction in energy at a given *f*, below 70 eV. All these features, and the modifications they imply, are natural consequences of a stochastic acceleration process favouring electrons in a selected band of energies, as some of us have recently demonstrated by numerical simulation[2] and a deterministic approach[2,3] to the problem.

The stochastic exchange of energy and momentum between electrons and packets of electrostatic waves of the lower-hybrid type is such a process. Electrons and wave packets can interact only when the phase and group velocities of the waves match those of the electrons. Although the wave fronts propagate relatively slowly and almost perpendicular to the magnetic vector, **B**, their projections parallel to **B** have velocities comparable to those of the electrons which, being strongly magnetized in the space plasmas under consideration, are constrained to flow in this direction. This resonance permits momentum and energy to be exchanged, the sense and rate of flow depending on the velocity distributions of the electrons and wave packets. Net flow is from waves to electrons when the electron distribution has a negative slope, as in the solar wind (*a* in the figure). Energy selection is expected because the process is so effective at low parallel velocities, where electron densities are highest, that the growth of waves is suppressed. A high-velocity limit can also be anticipated.

The concurrent observation that plasma waves near the shock appear as broadband noise extending from below the lower-hybrid frequency to the electron plasma frequency[4], is a positive, but not conclusive, indication of the presence of lower-hybrid waves in the region of acceleration. It remains to be seen whether the waves were of sufficiently high intensity. The free energy for generation of the waves derives in this interpretation



a, Electron velocity–space densities, *f* (relative scale), as functions of energy, in the magnetosheath of Mars (solid curve) and in the solar wind upstream from the planet (dashed curve), derived from measurements made by Phobos 2 on 1 February 1989. *b*, Electron velocity–space densities (in units of km^{-6} s^3), as functions of energy, observed at the centre (open circles) and edge (solid circles) on an electron stream producing a terrestrial auroral arc traversed by Skylark rocket SL1122 on 30 October 1973. The similarity with the martian magnetosheath distributions in *a* is striking and suggests to us that the same electrostatic-wave acceleration is responsible.

from the streaming energy of the solar-wind protons observed[5] from Phobos 2 to be retarded and heated at a location reached at 18:25 UT, intermediate between the observations of single- and double-peaked spectra.

Our explanation, that the lower peak is an unchanged property of the original solar-wind distribution and the higher peak is due to acceleration by lower-hybrid waves, is a direct application of a mechanism we have proposed[2,3,6-8] to account for electron acceleration in the terrestrial aurora, where very similar electron distributions can be observed (*b* in the figure), and in other instances of electron energization in space plasmas. The mechanism is of practical value in experimental nuclear fusion devices where the magnetic field of the current carried by electrons accelerated by lower-hybrid waves helps to contain the plasma, and electron energy communicated to ions serves to heat the plasma[9].

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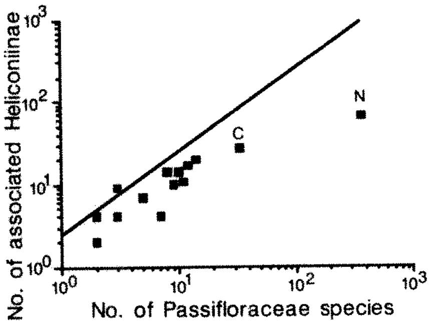
Fewer species

SIR—Erwin[1] obtained 1,200 species of beetle from the canopies of just 19 individuals of one tropical tree species, causing him to suggest that there could be 30 million arthropod species in the whole of the tropics, rather than 1.5 million, the approximate number of species then thought to exist. Stork[2] evaluated Erwin's assumptions and produced a range of values from 7 to 80 million or more species. But I calculate tropical arthropod diversity to be about 6–9 million species.

To produce their estimates, Erwin and Stork had to estimate the proportion of insect species specific to a given tree species. These authors ignored other insects as transients because these insects should be counted as genuine associates of other plants. But this categorization of insects is rather simplistic[2]. Many herbivores feeding on a given tropical plant species also feed on other plants in the same community[3], and insects are often specialists on different plant species in different places. If these insects are categorized as specific, they will be counted more than once in estimates of global diversity because the extrapolation procedure relies on multiplying the number of specific herbivores per plant by the number of plant species.

After eliminating transients, I corrected for the overlap in herbivore faunas between plants. A parameter (x) allows the number of herbivore species supported by a plant community (a) to be estimated from the number of species of herbivore per plant population (b) by the equation $a = x \times b \times c$, where c is the number of plant species in the community. For well-known communities, x can be calculated. For the herbivores of passion vines (*Passiflora*) at a site in Costa Rica (Sirena)[3], there are 30 herbivores for which adequate data exist (a), an average of 11.7 herbivore species per plant (b) and 9 *Passiflora* species (c), so x is 0.28. For the relatively host-specific Heliconiinae butterflies on *Passiflora*, estimates of x for four communities are 0.56 for the Arima Valley, Trinidad, 0.71 for Rincon, Costa Rica, 0.29 for Rio de Janeiro and 0.56 for Corcovado (including Sirena) in Costa Rica[4,5]. The mean x is 0.45. Without this correction, multiplying the number of plant species by the number of herbivores per plant produces an estimate of community diversity that is too high by a factor of about two (1/0.45 = 2.2).



Relationship between the number of Passifloraceae species recorded and the number of Heliconiinae species associated with those plants. Unlabelled points show data for single sites (from refs 4, 5, 8). C, combined data from Rincon, Arima Valley, Rio de Janeiro and Corcovado sites; N, total for neotropics (data from refs 6, 7); line, number of Heliconiinae species expected if a linear projection is made on the basis of the average number of Heliconiinae (2.73) on single *Passiflora* populations.

A parameter (y) that might allow the estimation of approximate global (or continental) diversity (d) from the number of species of herbivore per plant population (b) is given by $d = y \times b \times e$, where e is the world (or continental) number of plant species. Again, this parameter can be calculated for well-known groups. There are about 66 species of neotropical Heliconiinae butterflies[6], and a minimum estimate of the number of Passifloraceae in the American continents is 360 species[7]. From studies of 45 Passifloraceae populations drawn from 33 species[4,5], the average number of species of Heliconiinae per Passifloraceae population is 2.73. These values give an estimated y of 0.067. Without this correction, extrapolation from the number of Heliconiinae species on one Passifloraceae population to those associated with all American Passifloraceae would result in an overestimate by a factor of 15 (1/0.067; see figure). Tentatively, the overestimate can be partitioned into a within-community component of about 2, and a between-community component of about 7 (15/2.2).

Using $y = 0.067$ as a correction factor, I have recalculated diversity from Erwin's data and assumptions, except that I assume beetles to be 20% rather than 40% of tropical arthropod species[2]. This gives a value of 3 million arthropod species in tropical canopies, or 6–9 million if ground arthropods[2] are included.

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Horizontal flow of pore fluids

SIR—Hanor, in his calculation[1] of the density-driven subsurface movement of pore fluids near a salt dome on the Louisiana Gulf Coast, derived horizontal velocities of the order of 10 m per year at depths exceeding 1,000 m. These velocities are large for a sedimentary basin — Bethke *et al.*[2] cite values measured in cm per year — even in the geologically disturbed environs of a salt dome. Indeed, they are gross overestimates which stem from the erroneous assumption of hydrostatic equilibrium in the fluid flow. We repeated Hanor's calculations using a model that does not invoke the assumption of hydrostatic equilibrium and obtained velocities smaller by a factor of thousands.

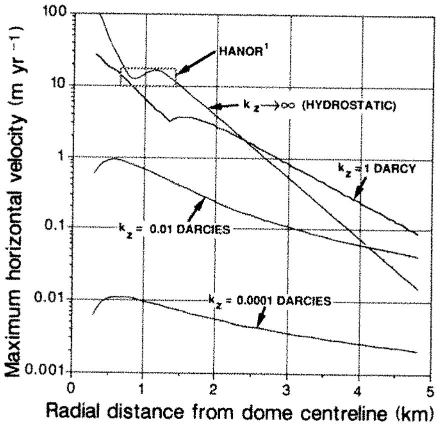
Although the assumption of hydrostatic equilibrium may lead to reasonable approximations of the absolute values of pressure, it is entirely inappropriate in predicting horizontal pressure gradients (horizontal components of "hydraulic force field" in Hanor's terminology) and the associated velocities of lateral density-driven flow in deep basins. Indeed, the hydrostatic assumption (also sometimes referred to as the Dupuit approximation) is tantamount to the neglect of the resistance to vertical fluid flow or, equivalently, to the assumption that the permeability is relatively large in the vertical direction (comparable to or greater than the permeability in the horizontal direction). This assumption is not valid for the geological system under consideration.

In reality, the vertical permeability in deep sedimentary basins, evaluated over thick sequences of alternating sands and shales, is not large, even next to a salt dome, as Hanor himself has more recently implied[3]. Moreover, convective fluid overturn, which is typical of density-driven fluid movement[1], will almost always be accompanied by a significant component of vertical flow, especially near an upward-tilting boundary such as a dome. Such a vertical flow will cause the system to deviate markedly from hydrostatic equilibrium.

In our calculations, we used a model of density-driven flow that combines the equation of continuity (conservation of mass) with Darcy's law for flow through porous media[4]. The model is not based on the hydrostatic assumption, and, moreover, recognizes that subsurface systems characteristically display highly anisotropic permeability behaviour when taken over thick sand/shale sequences, featuring very different values for permeability in the horizontal and vertical directions.

We sought to match as closely as possible Hanor's input parameters, including the imposed density distribution, the shape of the salt dome and values of all key physical properties. We used Hanor's value of 10^{-12} m^2 (1 darcy) for the horizontal permeability but varied the vertical permeability in the calculations. We also extended the calculations farther from the dome, using the reasonable constraint that the fluid density perturbation associated with the salt dome dies out gradually with horizontal distance.

As expected, our results agree with those of Hanor when the vertical permeability is set to a relatively large value, of the order of 1 darcy or greater (see figure). But with more realistic values of the vertical permeability between 10^{-16} and 10^{-17} m^2 (0.00001–0.0001 darcy), recently implied by Hanor as being reasonable for regions adjacent to salt domes, our calculated velocities decrease to between 0.1 and 1.0 cm per year. The calculations also show that the flow disturbance caused by density variations diminishes markedly with distance from the salt dome, indicating that this phenomenon is localized.



A comparison of our results with Hanor's data from ref. 1.

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HANOR REPLIES—Miller *et al.* misrepresent the purpose of my calculations[1] which were made to demonstrate that inversions in pore-water density arising from variations in temperature, pressure and salinity are of sufficient magnitude to drive kilometre-scale vertical overturn of subsurface pore waters at the Iberia salt dome, Louisiana Gulf Coast. But the calculations of Miller *et al.* support my hypothesis. The limitations of the approximation I used were completely understood and are specifically mentioned in my paper. Further, subsequent published calculations by me and others[3,5] using more rigorous techniques and more realistic boundary conditions have yielded comparable estimates for the range of the magnitude of hydraulic force which can be generated by spatial variations in pore-water salinity and temperature around salt domes.

Contrary to the impression that may be given by Miller *et al.*, I did not attempt to determine the fluid velocity field at Iberia because of the lack of adequate field information. To provide a preliminary order-of-magnitude estimate of fluid velocities which could result from the calculated forces, however, I did show that a horizontal hydraulic force of 50 Pa m^{-1} operating on fluids contained within a sand having an intrinsic permeability of 10^{-12} m^2 could induce velocities in excess of 10 m yr^{-1}. This choice of permeability was an appropriate reference value because massive sands at the Iberia dome are known from direct measurement to have such permeabilities and because Iberia is surrounded by a sand-dominated, not a clay-dominated sequence (see my Fig. 3). My more recent work[6] shows that even clay layers in the Louisiana Gulf coast can have effective vertical permeabilities two or three orders of magnitude higher than the values cited by Miller *et al.* as "more realistic", supposedly on my authority.

The detailed understanding of rates of deep fluid flow in the Gulf Coast will require more information than is generally available at present on the nature and local variability of driving forces and on the effects of media heterogeneity on permeability. The oversimplified conceptual and numerical modelling approach employed by Miller *et al.* is inadequate. Their conclusion, ". . .that the flow disturbance caused by density variations diminishes very markedly with radial distance from the salt dome, indicating that this phenomenon is very localized", is contradicted by published geological field evidence[7,8] clearly demonstrating that salt-dome-related solute transport is regional, not local. If Miller and his colleagues want to determine rates of subsurface fluid flow and solute transport in Louisiana, I recommend they begin monitoring the migration of the 7 × 10^9 litres of liquid hazardous wastes injected into the south Louisiana salt-dome province by their employer.

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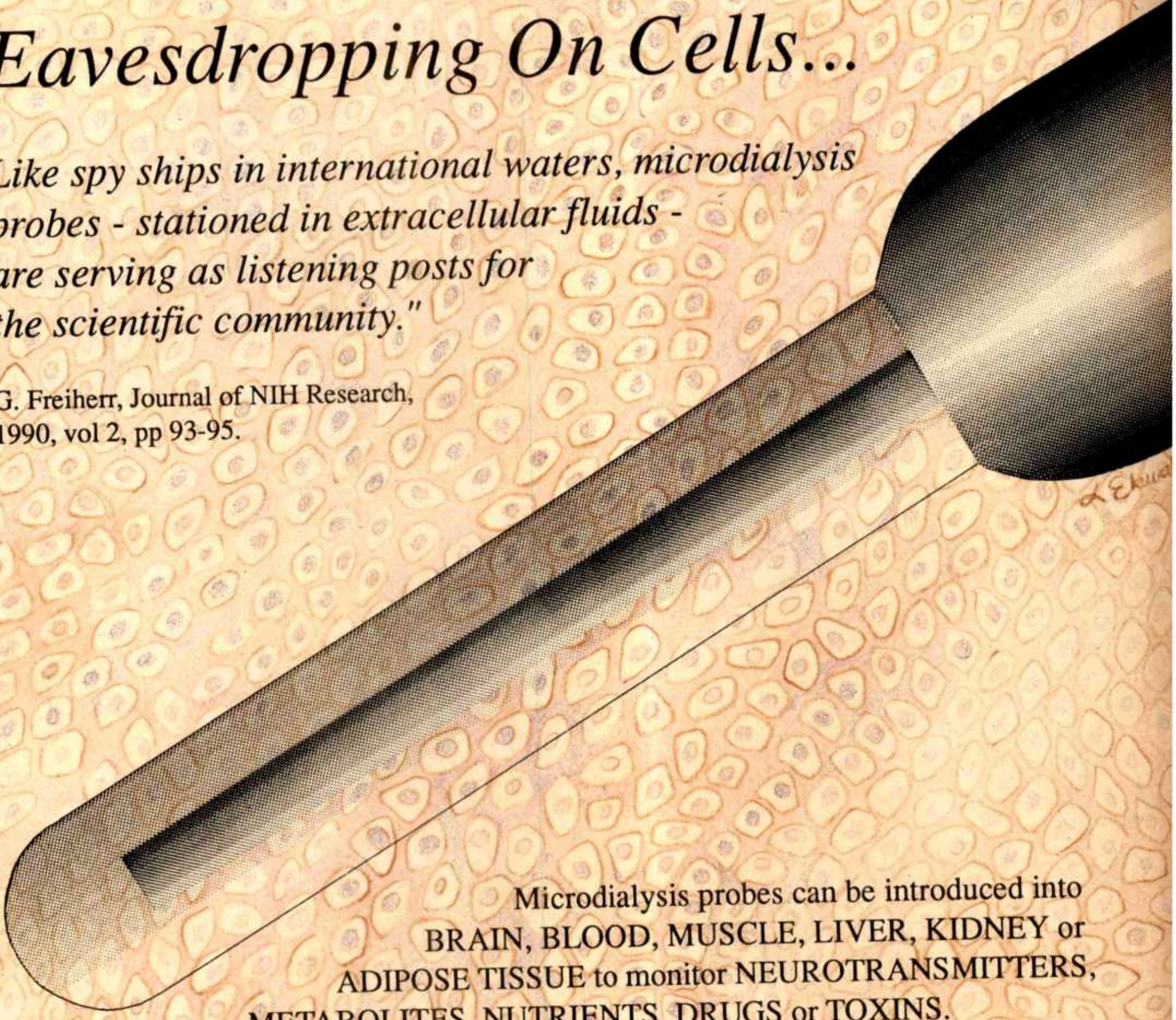

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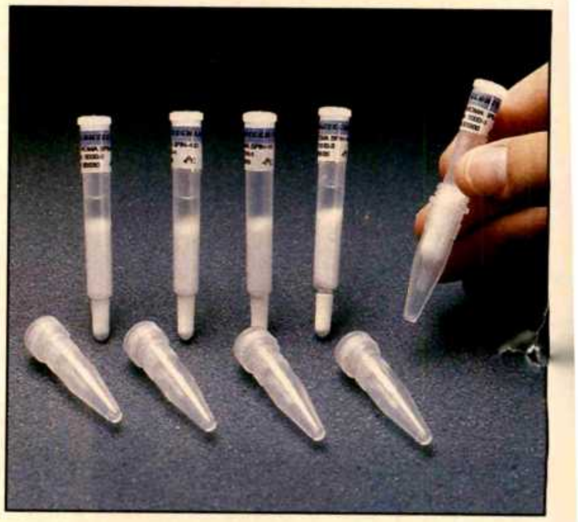

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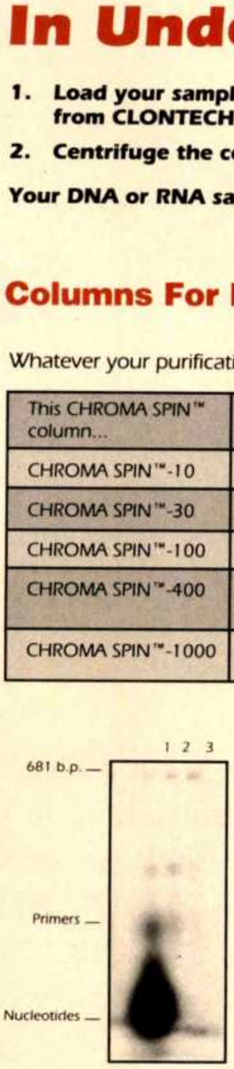

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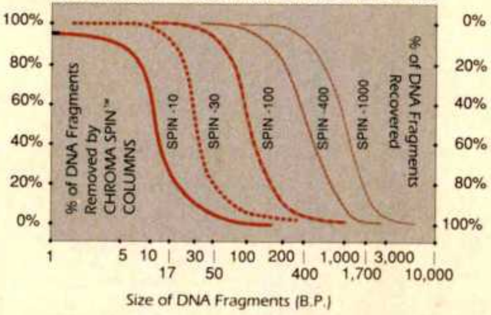

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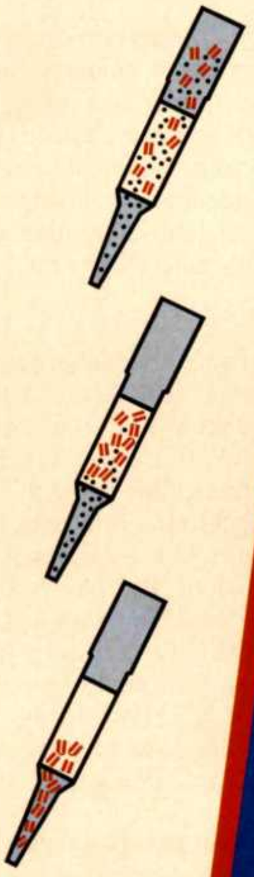

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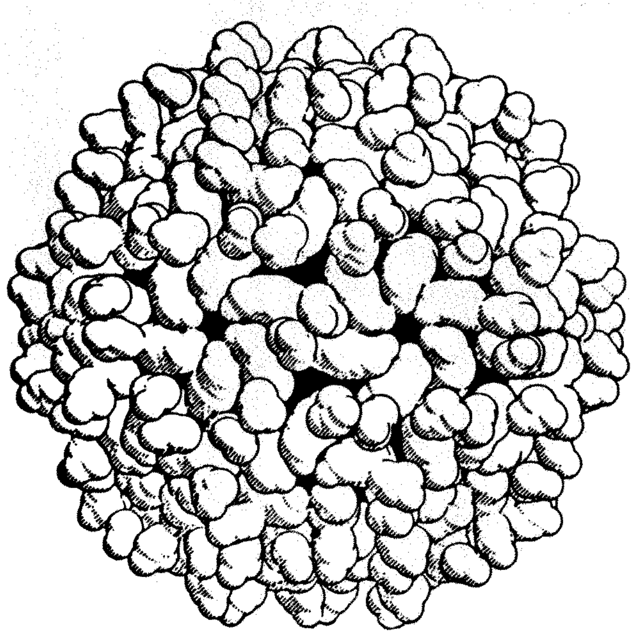

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Concepts in Viral Pathogenesis III

1989. XIX, 389 pp. 60 figs., incl. 5 color figs.
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The all new Concepts in Viral Pathogenesis III contains the widely praised format of presenting up-to-date information in pithy, easily read "mini-review" style and complements previous editions with contributions by leading international authorities on structure-function relationships, gene regulation, cell biology of viral infections, transgenic mice, expression of viral genes, retroviruses, and evolving concepts in viral diseases.

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1986. XIX, 425 pp. 31 figs. Hardcover DM 120,– ISBN 3-540-96322-7

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1984. XVIII, 409 pp. 26 figs. Hardcover DM 128,– ISBN 3-540-90982-6

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Less than total recall

Stuart Sutherland

Human Memory: Theory and Practice. By Alan Baddeley. *Erlbaum: 1990. Pp. 515. Hbk £25, $39.95; pbk £11.95, $19.95.*

A RECENT dictionary defines "Boxology" as "The construction and ostentatious display of meaningless flow diagrams as a substitute for thought". *Human Memory* contains some splendid examples. One has sixteen arrows connecting thirteen boxes, which are given such labels as encoding, recoding, conversion, cognitive environment, original engram and recollective experience. Even so, the author admits, the diagram is "a simplified version of the original". Just how the processes subsumed in each box are carried out, we are not told.

The main point of the diagram is that if anyone wants to recall material presented on a particular occasion he needs not only to remember the material but to have associated it with that occasion. Everyone already knows the word 'cold', but not everyone dragooned into being a subject in a psychologist's laboratory will recall that it was one of the fifty words read out to him there. Indeed, as Endel Tulving showed in a famous recent experiment on memory, the subject may fail even to recognize a word as part of the ensemble presented if given the wrong cue for retrieval, while being able to recall it if given an appropriate reminder of the occasion. (This finding was thought remarkable because normally recognition is much superior to recall.) The principle that one can remember what happened on a given occasion only if the events have been associated with it may well strike readers as rather obvious, but apparently it came as a surprise to workers in memory: it has been given the grandiose title "synergistic memory theory".

Baddeley does a competent job of expounding such experiments. He lays considerable stress on what he calls "theories", though for the most part they are vague speculations little better than dressed-up common sense. Work on human memory seems to make little progress, although this is not for want of effort, if one counts effort in man-hours rather than in intellectual exertion. There is still no clear answer to that old examination chestnut "Why do we forget?" Is it that the traces laid down in the nervous system (presumably changes in connectivity at synapses) decay spontaneously with time? Is it because material learned more recently changes the connections made in previous learning and hence makes it harder to retrieve the original material? Or do new traces fade more quickly than old ones so that if the material to be learned is similar to that already learned, the earlier material comes to overshadow the later? Are memories preserved only as a result of calling them to mind periodically? Or was Freud right in thinking that anything unacceptable to the ego is repressed and hence unavailable at a conscious level?

We do not know whether spontaneous decay of memory traces occurs, but we have known for many years that forgetting is increased by learning similar material either before or after that on which a person is tested. Baddeley is sceptical about repression, but points out that people asked to remember events from their early life remember a greater number of pleasant than unpleasant ones — but he fails to note that perhaps there really are more pleasant than unpleasant events. Not surprisingly, there is a tendency to recall more sad events when one is unhappy than when happy, and to colour one's interpretation of previous events with the current sadness. Oddly, there is little or no change in the number of happy events recalled depending on one's mood, though one may doubt how far laboratory manipulations really succeed in inducing either sadness or happiness.

Baddeley has curiously little to say about one of the most interesting recent discoveries in memory research. Explicit memory is the deliberate recollection of an experience, whereas implicit memory is the influencing of a response by a previous experience without the person knowing that he is being influenced. For example, if someone has recently read a list of words including the term 'clock', and is then asked to fill in the missing letters in 'clo--', he is much more likely to respond to 'clock' than 'close' even though he has no conscious recollection of having seen the word 'clock'. Such implicit memory differs from explicit memory in several ways, some of which are as follows. Changing the modality in which the word is presented, from, for example, vision to hearing, has no effect on explicit memory but decreases implicit memory. Asking subjects to process words at a high level, for example by thinking of their meaning rather than just their sound, improves explicit memory, but has no effect on implicit memory. The rate at which words are forgotten is very different (in rather complex ways) for the two kinds of memory. Learning new but similar material impairs explicit but not implicit memory. The two kinds of memory appear to be entirely separate because there is almost no correlation between success on the same item in an implicit and explicit memory task. Although Baddeley ignores these striking results, which are relevant to consciousness, he does mention that many amnesics with little or no explicit memory have comparatively intact implicit memory.

One unusual feature in what is basically a textbook on memory is the account of the application of work on learning to the

Land of the giants — early European travellers came back with over-awed and exaggerated descriptions of tropical rain forests; the engraving above (originally from *Flora Brasiliensis*, 1840) shows an artist's impression of a scene in the Atlantic coastal forest of Brazil and is reproduced from *An Introduction to Tropical Rain Forests* by T. C. Whitmore. This book describes the rain forests, their structure and dynamics, flora and fauna, and discusses their value to mankind and our impact on them. With examples taken from all parts of the humid tropics, the book is aimed at the general reader. Published by Oxford University Press, price is £35 (hbk), £16.95 (pbk). □

treatment of the mentally disordered. This section is, however, skimped and as Baddeley himself remarks "the conclusions that I draw . . . are, therefore, inevitably superficial". A second innovation is a chapter on treatment for amnesic patients, but the results are meagre. Persuading these people to associate names with visual imagery helps them remember the names, while their memory for the substance of a passage of text can be improved by the PQRST technique (*p*review the material; *q*uestion to find the salient points; *r*ead carefully; *s*tate the main features of the text; and finally *t*est oneself by checking memory for the text against it). Both techniques are of course known to help unaffected people a little, although they are based on common sense rather than psychological findings.

Although lacking in excitement, the book is for the most part competently written, provided you can bear the repeated misuse of the expression "as such". The same cannot be said for the index, which is a disgrace. The contents are not memorable: only readers adept at the PQRST technique are likely to commit them to mind. ☐

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition, Department of Experimental Psychology, University of Sussex, Brighton BN1 9QG, UK.

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Forcing the tissue

R. H. T. Edwards

Skeletal Muscle in Health and Disease. A Textbook of Muscle Physiology. By D. A. Jones and J. M. Round. *Manchester University Press: 1990. Pp. 221. Hbk £29.95, $49.95. Pbk £9.95, $17.95.*

For hundreds of years, skeletal muscle has presented an intriguing challenge to those interested in understanding the generation of force and other physiological and metabolic properties of this tissue. The principal models that are the basis of our understanding of skeletal muscle structure, function and chemistry are largely derived from studies of muscle isolated from various animals. Over the past 20 years, the growth of methods of chemical analyses of needle biopsy samples and electrical stimulation techniques has been quite remarkable, allowing human muscle to be studied to something approaching a degree of precision similar to work on isolated muscle. The authors of this book have distilled in their short volume a wealth of experience in developing and applying techniques for the study of human muscle in health and disease.

The strength of the didactic account of the subject given here lies in the fact that both authors are laboratory researchers who have worked in clinical departments, with the result that discussions on basic mechanisms are extended to man by, for example, descriptions of a cross-section of muscle obtained by X-ray computerized tomography and a wealth of (admittedly monochrome) photomicrographs of muscle in various pathological states. The account of muscle structure and function is greatly helped by illustrations of the structure and inter-relations of particular cellular components. Classical muscle physiology is concisely but authoritatively covered with a profusion of specific examples on different isolated muscle preparations.

The growth and development of muscle and the implications for athletic performance lead on to an up-to-date account of modern concepts of physical training. This account is particularly interesting in view of the authors' own observations on the relationship between cross-sectional area measured by X-ray tomography and the increase in maximal isometric strength with training. Force has long been known to be proportional to cross-sectional area of muscle, but it has not been at all clear how muscles can become stronger with little or no increase in cross-sectional area. The authors suggest that tethering by new connective tissue increases force through a reduction in effective fibre length. Also of interest to athletic performance is the section on the adaptations for endurance exercise, which covers well-documented work carried out in Scandinavia, the United Kingdom and in North America on the energy supply processes for muscular activity.

The sections on fatigue and pain are those in which the authors make their most original contributions. Their account of the complexities of the mechanisms underlying fatigue in isolated muscle and in human subjects is commendably clear and concise. Vigorous muscular activity is always at risk of causing muscle damage or pain, and it is refreshing to see the systematic analysis of the mechanisms underlying these based on experimental observations.

In the final section, the authors consider the reactions of muscle not only to mechanical damage but also to the consequences of disease. Muscle as a specialized tissue responds to insult in relatively few ways. What is seen as compatible with a particular clinical diagnosis comprises both primary features and (often more obviously) secondary adaptations to damage or compensatory use. Jones and Round provide examples of these adaptive responses obtained from biopsies from people with Duchenne muscular dystrophy, spinal muscular atrophy and inflammatory muscle diseases.

The value of percutaneous muscle biopsy as a tool to give a 'life vision' (*Bios, Opsis*) to supplement the physiological and metabolic study of human muscle disease is obvious. The recent, rapid increase in our knowledge of the muscular dystrophies obtained from molecular-biology methods is summarized here for Duchenne and Becker muscular dystrophies, together with reference to the possible physiological role of the cytoskeletal protein dystrophin which is absent in people with these types of muscular dystrophy.

This text is likely to become a standard for student courses on cell biology, physiology, sports sciences and research methods in physiotherapy. The book deserves a wider readership, however, as an exciting introduction to the rapidly expanding field of muscle biology and how it has influenced our understanding of human muscle performance and the pathophysiology of muscle disease. ☐

R. H. T. Edwards is Professor of Medicine at the University of Liverpool, PO Box 147, Liverpool L69 3BX, UK.

■ Some of the techniques mentioned above are covered in *Scanning Electron Microscopy, X-Ray Microanalysis, and Analytical Electron Microscopy*, new from Plenum. This laboratory workbook, by Charles E. Lyman *et al.*, provides exercises for students developed at courses held annually for the past few years at Lehigh University. Spiral bound, price is $29.95. ☐

Monod and stereo

John Galloway

Mechanisms of Cooperativity and Allosteric Regulation in Proteins. By Max Perutz. *Cambridge University Press: 1990. Pp. 101. Pbk £11.95, $17.95.*

'ALLOSTERY' does not feature in Macmillan's *Dictionary of the History of Science* — a rather striking omission. But Ptolemy's spheres do. Possibly with a touch of irony, Max Perutz likens the appeal of symmetry for Jacques Monod, who formulated the idea of allostery, with the aesthetic attraction exercised by the spheres. Thus Perutz links second-century astronomy with twentieth-century molecular biology through one of the principles that threads its way through all science. It turns out that symmetry is not necessarily the central issue in allostery (although it is sometimes important) any more than helicity is necessarily the central issue in the structure of DNA, although these issues are the features most people know about in each case.

I mention allostery and DNA together because if DNA was nature's first secret, allostery was certainly its second, as Monod claimed. Neither could be called a secret today. Perutz's book describes how for allostery the word has been made flesh using the inexorable methodology of X-ray crystallography. Be warned though, the 'book' is actually a 100-page article reprinted from *Quarterly Reviews of Biophysics*, and will be tough going for most readers. Take with a small pinch of salt the blurb's claim that the book may "appeal to anyone wanting to understand the interplay between chemistry and motion that is the very essence of life". It may, but you will have to work at it.

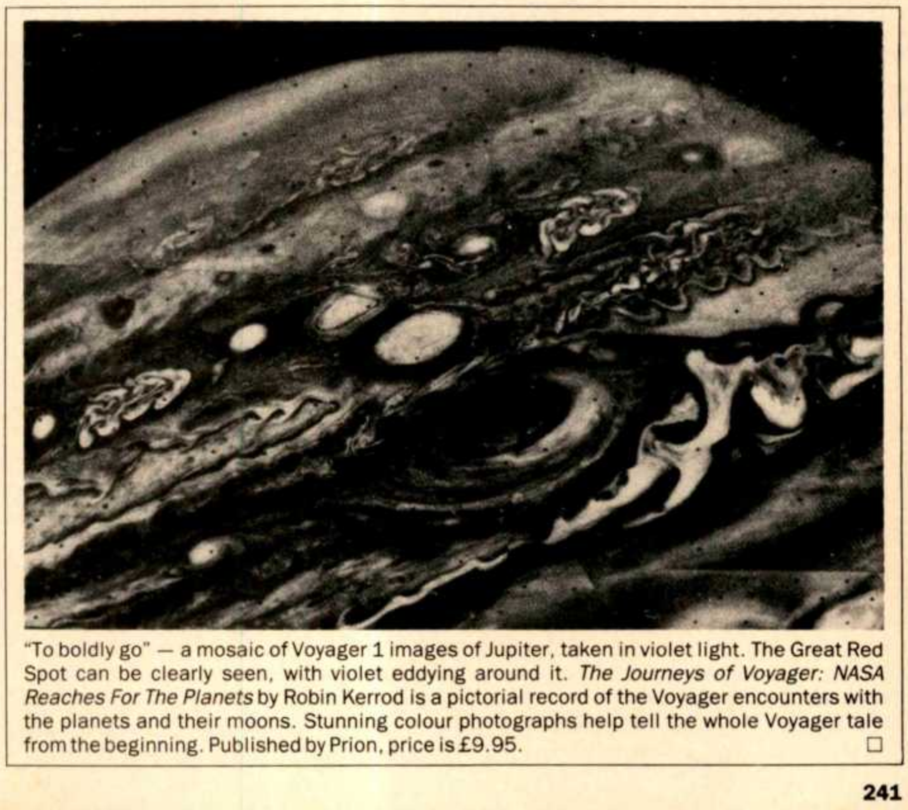
What exactly is allostery? Some proteins are now known to have two three-dimensional structures, and an allosteric transition is the switch between one structure and the other. Monod, and those with whom he worked closely on the idea of allostery in the early 1960s, were interested in the idea of metabolic interconnectedness — that critical metabolic steps might (must) be controlled by "metabolites acting as physiological signals rather than as chemically necessary components of the reaction itself". The systems exercising such control seemed very diverse, but could they be explained by a few simple general principles?

Monod and his collaborators proposed that the control was mediated by proteins with special properties. In general, they thought these proteins possessed two receptor sites — an active one responsible for the protein's actual biological activity and binding the substrate, and an allosteric site more or less remote from the active site and binding an effector molecule, a binding that caused a (reversible) change in the protein structure and altered the properties of the active site. More particularly, Monod *et al.* suggested that the most interesting instances of allostery would involve cooperativity between identical sites. Cooperativity seemed to demand some sort of repetitiveness in the protein's structure, in other words a symmetrical arrangement of identical subunits (or an elaboration on such a theme). Transition in the tertiary structure of the subunits would be translated into a corresponding transition in the quarternary structure of the whole molecule between an inactive state in which the subunits were tightly bound — which Monod *et al.* called T (for tense) — and an active state, R (for relaxed). Taken to extremes, a relaxed state is a state of dissociation of the protein into its individual subunits.

Perutz's review was stimulated by the spate of enzyme and other protein structures that were published in 1988 in which both R and T structures were described. Perutz describes the structures of the molecules and the way they work in great detail, the sort of detail we now expect crystallography to provide. The predictions of Monod *et al.* are brilliantly borne out. "The subunit contacts of haemoglobin, phosphofructokinase and aspartate transcarbamylase, are very clearly designed to allow only concerted transitions between alternative quaternary structures".

Today we know very well what presumably was not known in the early 1960s — that proteins combine elements of both rigidity and flexibility in their structures, both exploited in the biological roles they play. Looking back to those early days it is striking how structural theory was running ahead of experiment. Although the Monod school was interested in enzymes, its seminal paper, published in 1965 in the *Journal of Molecular Biology*, actually opens with a discussion of haemoglobin. Perutz in his new book praises the boldness and imagination of "an analogy between feedback inhibition in aspartate transcarbamylase and threonine deaminase and inhibition of haemoglobin by hydrogen ions" which led to the paper. And indeed the first third of his review describes haemoglobin and its close molecular relations. Interestingly, Perutz's own papers do not seem to refer to Monod's idea before 1970, when he had obtained good high-resolution data on both forms of the molecule. He wrote in *Nature* that year "For purposes of comparison, haemoglobin can be compared to an enzyme made up of two different pairs of subunits with oxygen as the substrate, haem as the enzyme and H^+ and DPG as allosteric effectors".

The interplay between rigidity and flexibility in proteins makes it tempting to draw a comparison with the theory of the structure of the spherical viruses. In the years leading up to Monod and collaborators' first papers on allostery in the early 1960s, Aaron Klug and Don Caspar were propounding their theory of the structure of the spherical viruses based on Buckminster Fuller's geodesic domes. The theory included the necessity of quasi-equivalence — seemingly identical proteins being arranged non-equivalently in the viral coat and demanding flexibility



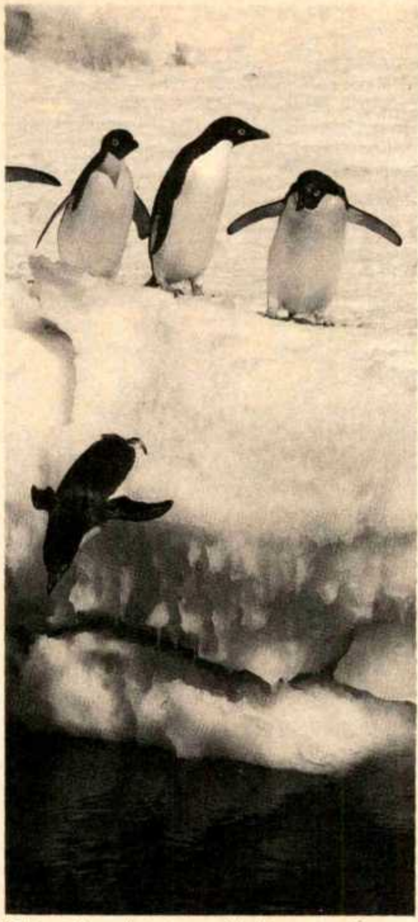
"To boldly go" — a mosaic of Voyager 1 images of Jupiter, taken in violet light. The Great Red Spot can be clearly seen, with violet eddying around it. *The Journeys of Voyager: NASA Reaches For The Planets* by Robin Kerrod is a pictorial record of the Voyager encounters with the planets and their moons. Stunning colour photographs help tell the whole Voyager tale from the beginning. Published by Prion, price is £9.95. ☐

in the way they bonded to one another.

In 1964, electron micrographs of human-wart, simian 40 and polyoma viruses were published, showing pretty convincingly that this theory was correct. In a report in *Science* that year by Victor McElheny, Perutz was quoted as saying "The results had surprised us all. The theory was pretty far fetched, you know." Why far fetched? The obvious answer is that protein crystallographers did not think that protein structures could be flexible and hence provide a mechanism.

It does seem an extraordinary coincidence, looking back, that theory and experiment were running on such parallel tracks. Of course what really matters is how luminous an idea is, and by any standards allostery is pretty luminous. An elegant idea shows how the self-regulation of very complicated systems is possible. Whether Monod's school thought that its idea could be realized only through its specific model is not clear — at least to me. What Perutz's review brings home is the power of crystallography to show how the idea is realised in concrete terms. Detailed descriptions free us to some extent from the reliance by theory on metaphor or analogy and over simple preconceptions. Whether the descriptive richness is yet quite the same thing as a full understanding remains to be seen. ☐

John Galloway is at the Cancer Research Campaign, 2 Carlton House Terrace, London SW1Y 5AR, UK.



Taking the plunge — Adelie penguins are bold and curious birds, often unable to resist the temptation to follow should one of their colleagues stroll along the beach or dive into the sea. Every October the penguins return to their rookeries around the coasts of the Antarctic, sometimes walking or tobogganing over as much as 96 km of ice. *Birds in Focus* by Mark Cawardine is filled with more than 200 superb photos and many fascinating facts about birds of all kinds. Published by Salamander Books, price is £16.95. ☐

Watching brief

Jeremy J. D. Greenwood

Waterfowl Ecology. By Myrfyn Owen and Jeffrey M. Black. *Blackie: 1990. Pp.194. Hbk £30, pbk £13.95. Published in the United States by Chapman and Hall. Hbk $71.50, pbk $35.*

Ducks, geese and swans — the avian family Anatidae — are the particular favourites of many birdwatchers. Most of them are relatively large and live on water or in open terrestrial habitats where they are easy to observe, often in large numbers. Furthermore, they are easy to tame and they appear to behave fairly normally when rendered flightless by wing-clipping, so they make good subjects for both private collections and public displays. There can be few children in Europe and North America who have not fed the ducks or swans on their local pond. Wildfowl also make good quarry, which not only means that there are many hunters with a passionate interest in them but that they are the basis of a huge industry serving those hunters. Others shoot — or otherwise seek to control — some species (especially geese) because of the damage they do, or appear to do, to crops. And since the perceptions of birdwatchers, wildfowlers and pest-controllers may be different, these birds and their ecology are often the subject of controversy.

So a book on wildfowl ecology should be useful. One written by Myrfyn Owen and Jeffrey Black, of the Wildfowl and Wetlands Trust, ought to be good. This result is both useful and good. It covers not only the whole of wildfowl ecology in the strict sense, with chapters on food and population dynamics, but also other aspects of the birds' natural history (breeding biology, social behaviour and movements). It does so systematically and in relation to general evolutionary and ecological principles. It is up-to-date in outlook and content, and lively to read. Because the family comprises a limited number of fairly similar species, the authors have been able to be comprehensive without being tedious or overwhelming; yet there is sufficient diversity to provide useful contrasts.

Blackie's Tertiary Level Biology Series, to which this book belongs, is designed for specialist courses at the advanced undergraduate level. Although there are unlikely to be many such courses devoted exclusively to wildfowl, the popularity of the group is such that this book will provide useful background reading for broader courses. Students will find here a wealth of solid examples, from species with which they may be very familiar, to illustrate the general principles that they have learned from more general texts or courses in ecology, wildlife management or ornithology. The book will also be a useful tool for the education of new graduate students embarking on wildfowl research, for academic ecologists working on other organisms who wish to broaden their knowledge, and for wildlife managers who wish to know more about a group of birds to which so much management effort has been devoted.

Yet *Waterfowl Ecology* left me feeling disappointed on two counts. Although the material is presented firmly in a framework of general principles, these principles themselves are rarely brought into clear focus. The book is certainly not 'ecology, as illustrated by waterfowl'. But then it shouldn't be: the reader should know the principles before coming to this book. My disappointment was therefore unjustified. Nonetheless, I have a lingering feeling that the chapter on population dynamics should have had a full population model for at least one species and certainly a mention of key factor analysis.

My second disappointment came at the end of the chapter on conservation and management. Given the immense experience of wildfowl management, I wished that the chapter had been twice as long. But that would have unbalanced what is now a well-balanced book and the authors were right to keep each part of their subject within bounds.

Wildfowl provide some of the finest birdwatching memories: the crooning of courting eiders, the wild calls of newly arrived whooper swans passing over a Scottish firth at night, the sky-music of a distant skein of barnacle geese moving over snowy mountains, the clamour of pinkfeet tumbling out of the sky to their roosting water, and the delight of small children at the ducklings on the village pond. Owen and Black provide a substantial contribution to an intellectual appreciation that will complement such aesthetic experiences. ☐

Jeremy J. D. Greenwood is Director of the British Trust for Ornithology, Beech Grove, Station Road, Tring, Hertfordshire HP23 5NR, UK.

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As commercial production of therapeutic monoclonal antibodies becomes a reality, researchers and scientists require accurate, up-to-date information to keep abreast of this rapidly moving field. There are few reliable sources of information available, particularly in one convenient publication. **Therapeutic Monoclonal Antibodies** fulfills the need for a single reference covering the latest advances in both preclinical and clinical work on monoclonal antibodies. Written by an international team of leading experts, this exhaustive work details the latest progress made in the clinical application of monoclonal antibody technology.

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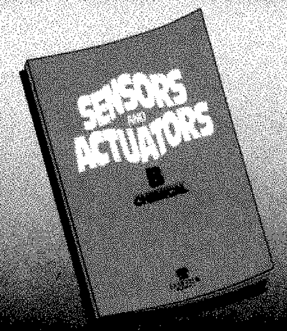

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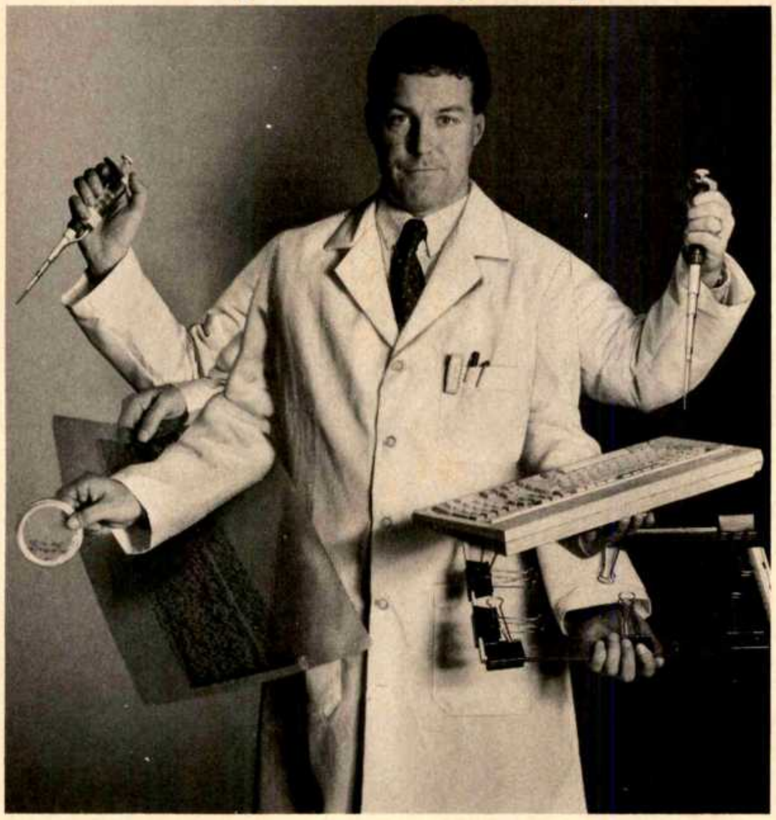

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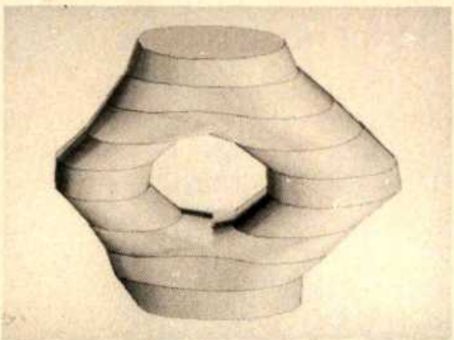

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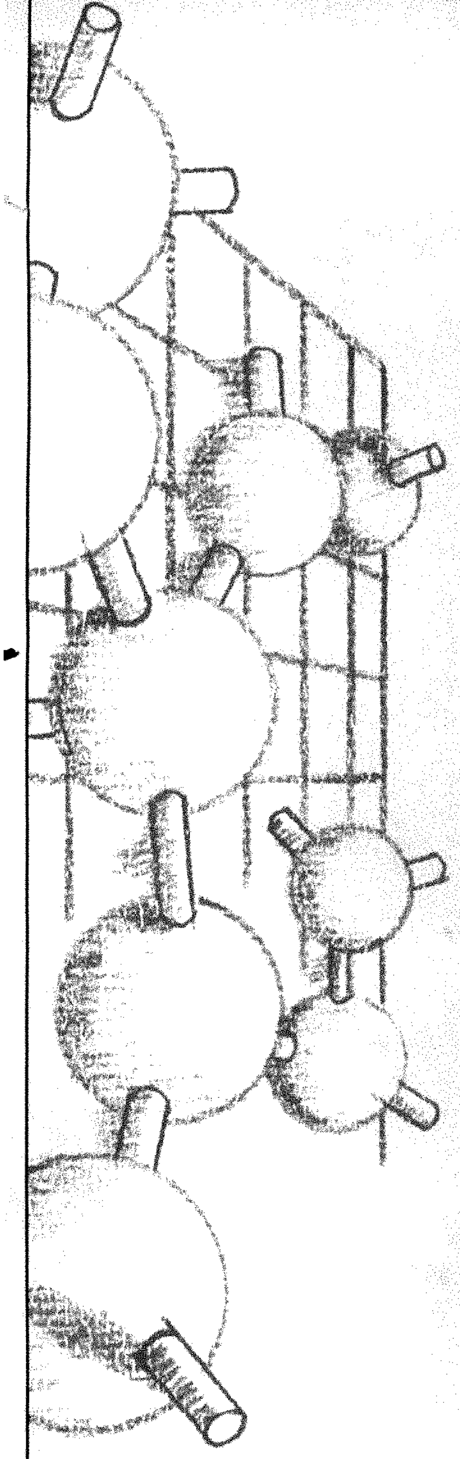

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DU PONT

Computer modelling of solid-state inorganic materials

C. R. A. Catlow & Geoffrey D. Price

Computational methods can now make detailed and accurate predictions of the structures of inorganic materials. Electronic-structure calculations are being performed for increasingly large systems, and simulation methods based on 'effective potentials' may now be used to model complex materials. One of the most exciting fields of application is mineralogy, which poses a major challenge to the predictive capacity of contemporary computational techniques.

THE past 10 years have seen significant progress in our ability to undertake accurate and increasingly predictive calculations of the crystal structures and properties of solids and of the defects and impurities that commonly control important aspects of their behaviour. It is now recognized that modelling techniques have the potential to contribute to the design and improvement of materials with specific characteristics and that they may be used to predict the properties of solids under extreme conditions, a topical example being the behaviour of minerals under the very high pressures and temperatures of the Earth's lower mantle. The development of the field has also been assisted by the recent enormous expansion in computer power, which has enabled calculations to be performed on complex materials whose study by theoretical techniques would have been inconceivable even five years ago.

The materials studied most extensively by theoretical methods are metals[1], for which band-structure techniques have led to detailed knowledge of their electronic structure and some success in prediction of their structures. Here we will be more concerned with inorganic non-metallic crystals, with an emphasis on the modelling of structural, elastic, lattice dynamical and thermodynamic properties. We shall summarize the main techniques employed and illustrate their use by several recent examples, giving particular emphasis to the field of mineralogy, which poses some of the most exciting challenges to the current methodologies. Our aim is to answer the following questions. How well can existing techniques model the structure and properties of inorganic solids? What are the main problems and limitations? What future areas of application can be envisaged?

One can distinguish two general classes of techniques: electronic-structure calculations, which attempt, at some level of approximation, to solve the Schrödinger equation for the system; and simulations, which make use of 'effective potentials' that describe the interatomic forces in the system and which may be used to predict structural and dynamical properties of the solid using essentially classical techniques. Interactions between the two approaches are increasingly strong and indeed many of the most important future areas of application will require concerted use of both types of method. We shall describe both approaches, ut with a degree of emphasis on simulations.

lectronic-structure calculations

wo approaches may be adopted in attempting to solve the chrödinger equation in solids. In the first, a cluster of atoms presenting the solid is studied; in the second, periodic boun-ary conditions are applied to the primitive unit cell (or to a upercell). Both approaches are used widely in contemporary studies.

Cluster calculations. These methods, which have been in use for over 20 years[2], are simple in concept. Once a suitable cluster has been defined, standard quantum-chemistry techniques can be used to identify the geometry of minimum energy. By examining the cluster wavefunction, the electron density distribution and other aspects of the bonding may be investigated; and by varying the geometry of the cluster, interatomic potentials and force constants may be deduced. Indeed, such calculations are being used increasingly to provide the potentials used in simulation studies.

For cluster calculations, there are three choices to be made:

(1) Choice of cluster size. Obviously the larger the cluster, the more closely are the calculated bonding properties likely to resemble those of the real infinite crystal. There will generally be a trade-off, however, between the cluster size and the level of sophistication of the calculation[3].

(2) Choice of cluster termination. As the cluster is a fragment of the solid, the peripheral atoms either will have unfilled valence shells ('dangling bonds') or will be charged. It is usually desirable to saturate dangling bonds and if possible to 'embed' the cluster by representing in some way the effects of a surrounding lattice. To achieve the former, hydrogen atoms are commonly used, although this may be unnecessary for more ionic systems in which the peripheral atoms may simply be closed-shell ions. See refs 2, 3, 4, 6 and 8 for further details and discussion.

(3) Choice of quantum-mechanical technique. Many recent cluster calculations on solids have used Hartree-Fock methods and computational techniques developed to describe the quantum chemistry of small molecules. These methods use a molecular wavefunction represented as a linear combination of atomic orbitals. Electron-electron interactions are calculated as Coulomb and exchange interactions, and a self-consistent wavefunction is obtained using an iterative procedure. The techniques range from semi-empirical procedures such as CNDO and MNDO, which ignore or parameterize many of the overlap integrals, to *ab initio* methods in which all integrals (above a threshold energy) are evaluated.

The principal shortcoming of the Hartree-Fock method is its neglect of the effects of electron correlation. These can be included by the use of multi-determinantal wavefunctions; but the computational cost is considerable. Valence-bond methods build correlation more directly into the wavefunction, and cluster calculations for solids using such techniques have been reported[8]. An attractive and increasingly popular approach is to use the local-density approximation (LDA) of density functional theory. This is based on the demonstration by Hohenberg and Kohn[9] that the ground-state energy of a system is a unique function of its electron density (strictly, the energy expression is a functional, as the electron density is itself a function of position). A self-consistent density is calculated using the variational principle (that is, by minimizing the total energy). Electron exchange and correlation energies can be evaluated by inserting the local density at a given position into the expressions appropriate for a uniform electron gas at that density. It is difficult to assess exactly the magnitude of the error introduced by this local approximation; but the method does involve the correlation energy directly, and it is enjoying considerable success in modelling the ground-state properties of solids.

Moreover, the efficiency of the method in locating minimum-energy geometries has been greatly enhanced by the work of Car and Parrinello[10], which borrows ideas from the molecular-dynamics simulation technique. Cluster calculations have been reported that use LDA methods[11], but most applications involve periodic systems (see below).

The question of the relative merits of LDA and Hartree-Fock methods is controversial. A good recent review is given by Jones and Gunnarsson[12]. LDA methods unquestionably have the advantage of being computationally cheaper, and therefore permit calculations at the *ab initio* level on larger systems. Their inclusion of correlation effects directly is a further substantial advantage. For these reasons, LDA methods have been used more widely than Hartree-Fock methods in recent work on solids. The latter methods do, however, have a substantial part to play, as is clear from the recent studies of SiO_2 described below.

Periodic boundary calculations. Both Hartree-Fock and LDA methods may now be used routinely to calculate the energies of an infinitely and periodically repeated unit cell. The most notable work using periodic-boundary Hartree-Fock calculations is that of Pisani and co-workers[13], whose CRYSTAL code is now making major contributions to our understanding of bonding in solids; examples of its application to SiO_2 and silicates are given below. LDA methods have been applied extensively to semiconductors; below we discuss recent applications to crystalline silicon and silicate perovskites.

Simulations

In simulation methods, the detailed effects of electronic structure are subsumed into 'effective potentials'. The type of potential model used depends on the class of solid. Most of the properties of ionic solids may be modelled successfully by adding short-range pair potentials to the Coulombic forces acting between the ions, and by treating ionic polarization using the shell-model formalism developed by Dick and Overhauser[7]. For semi-ionic solids such as SiO_2 it is necessary to extend the treatment to include many-body terms, which may be treated by incorporating specific angle-dependent forces (depending, for example, on the O—Si—O bond angles). Alternative many-body formalisms include the 'triple-dipole' terms used originally by Axilrod and Teller[14], which have been incorporated successfully into model potentials for the silver halides[15]. Approaches to modelling covalent solids are often based on valence-force potentials, which incorporate bond-stretching and bond-bending terms.

The most important step in any simulation is the parameterization of the potential. Empirical fitting procedures employing model compounds have been widely used. There is, however, an increasing incentive to develop reliable methods for calculating directly the short-range component of the potential. The electron-gas methods of Wedepohl[16] and Gordon and Kim[17] have been used extensively and successfully by Mackrodt and coworkers[18] to derive potentials for ionic solids. Quantum-mechanical cluster calculations are, however, being applied increasingly to the development of interatomic potentials; the strategy is to vary the geometry of the cluster in a systematic way and to fit the resulting potential-energy surface to an effective-potential model. There are many examples of such calculations, including the recent work of Lasaga and Gibbs[19], who performed detailed studies on the $Si(OH)_3{-}O{-}Si(OH)_3$ molecule in order to derive potential parameters for SiO_2, the modelling of which will be discussed in greater detail below.

Considerable progress has been made in developing interatomic potentials for metals. Here, tight-binding calculations have been found to justify some of the empirical approaches, and there is growing use of 'embedded atom' models based on effective-medium concepts. Several discussions can be found in ref. 1, and Finnis *et al.*[20] have provided a good review.

Simulation codes are generally based on one of three techniques: energy minimization, molecular dynamics and Monte Carlo methods. Energy minimization simply involves generating the configuration of lowest potential energy for a given potential model. Molecular dynamics methods, which include kinetic energy explicitly in the simulation, are widely used to study fast diffusion processes in solids[21] and properties of materials at high temperatures, for which the techniques that assume harmonic lattice vibrations are no longer valid. These methods follow molecular motions by solving Newton's equations of motion in discrete time steps. The Monte Carlo technique is essentially a method of computational statistical mechanics in which changes in the structure are successively made at random, with the configurations being accepted or rejected depending on an energy criterion. It is used most appropriately for disordered systems, recent examples being the study of sorption in porous solids[22] and vacancy distribution in heavily doped fluorite oxides[23].

A particularly successful simulation technique for studies of the solid state is the Mott-Littleton[5] method for studying defect properties. The approach combines energy minimization of a localized region of the lattice around the defect with a continuum treatment of the polarization of the remainder of the crystal. Reference 24 gives a comprehensive review.

Simulation methods are in general computationally cheaper than electronic-structure techniques. Moreover, they can yield information on time-dependent and thermodynamic properties

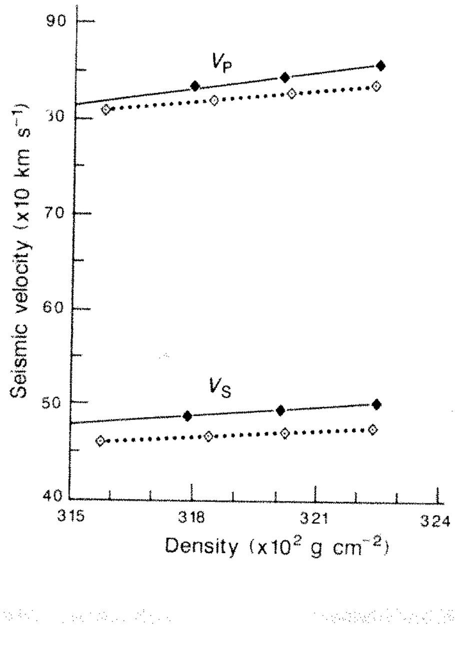

FIG. 2 Predicted (dotted line) and observed (solid line) P- and S-wa velocities of forsterite as a function of density[60].

which are inaccessible at present from direct quantum-mechanical formulations. For the foreseeable future, they will be the natural method for investigating large and complex systems. And they will always be the more appropriate method for investigating those problems for which the essential physics can be described by an effective-potential model.

We shall now describe some recent applications of many of these techniques to problems of topical interest.

Electronic-structure studies of silicon

The ability of *ab initio* electronic-structure techniques to make detailed and accurate predictions of the properties of crystalline solids is shown most clearly in studies of silicon. Yin and Cohen[25] used *ab initio* LDA techniques to show that the diamond structure has the lowest energy, as found experimentally, and that the calculated lattice constant and compressibility are very close to (within 1% of) the experimental values. They also studied the variation of stability with pressure, demonstrating that the metallic, white-tin structure becomes stable at 100 kbar and that a hexagonally close-packed phase forms at 400 kbar. The latter prediction has been verified experimentally.

This work and that of others shows clearly that it is possible to predict, from first principles and without the use of parameterized models, the structures and properties of structurally simple materials containing light atoms. The use of pseudopotentials (which represent the effects of core orbitals in a simple and economical way) should extend the scope of such calculations to more complex materials containing heavier atoms[26].

Simulation of SiO_2

Most modelling studies of SiO_2 have been based on simulations using effective potentials, although a valuable set of Hartree–Fock calculations using the CRYSTAL code with periodic boundary conditions have been reported by Dovesi *et al.*[27]. That study reproduced the structure of the material well; greater precision has been achieved in a recent study by Nada and coworkers (to be published). LDA methods (employing the Car–Parinello approach) have been equally impressive in modelling the structures and properties of cristobalite[28]. Several simulations of SiO_2 have been reported recently, with goals ranging from a simple description of the equilibrium structures of the SiO_2 polymorphs[29–31], through a description of the equation of state of quartz and its polymorphs[32–34], to studies of the behaviour of amorphous silica[35,36] and even the rheological properties of quartz[37].

The polymorphs of SiO_2 pose a major challenge to modellers, because their bonding is neither classically ionic nor ideally covalent. Hence, simple pairwise-additive ionic models are unable to describe the structural and elastic features of these phases, whereas models that describe short-range interactions and the directionality of the Si—O bond but that ignore the longer-range components fail in their description of the lattice dynamics and thermodynamic properties. This has led to the development of empirical models that incorporate both ionic features and covalent or short-range, angle-dependent terms[29–31]. Arguably the first, and possibly still the most versatile potential to describe satisfactorily the structural and thermodynamic properties of SiO_2 phases was developed in 1984 by Sanders and coworkers[29]. In this potential, Si and O are assigned full, formal-valence charges, but the directionality of the Si-O bond is modelled by introducing a bond-bending term. The polarizibility of the oxide ion is modelled by a shell-model potential. The model reproduces not only the structural and elastic properties of quartz[36] and other framework silica phases[38] but also their spectroscopic and thermodynamic properties. In addition, the potential can be used to describe the behaviour of the more complex magnesium silicates[39–41].

Other potential models, both empirical[32] and based on quantum mechanics[19,31], have also been developed; but in general they have not been critically assessed against the model of Sanders *et al.*, so it is difficult to determine whether they actually represent improvements. Thus, for example, Gibbs and Lasaga[19,31] have performed quantum-mechanical simulations on 'silicate molecules' that can be considered as fragments of silicate minerals. Their calculations involve a Hartree–Fock energy-level solution of the Schrödinger equation, and provide a potential-energy surface that describes the interatomic interactions within the fragment. These calculations are successful in modelling the known behaviour of true silicate molecules (such as disilic acid, $H_6Si_2O_7$), and the resulting potential-energy surfaces have been fitted both by a simple ionic model and by an essentially covalent model. In common with previous studies, these workers find that a two-body ionic model is unsatisfactory and conclude that an angle-dependent, covalent model is preferable.

The importance of many-body interactions in determining the behaviour of silica phases has also been highlighted by the use of modified-electron-gas (MEG) approaches. A problem of the MEG approach is that the O^{2-} ion is not stable in isolation, and must be stabilized by being contained within a charged sphere (the so-called Watson sphere) which simulates the stabilizing effect of the 'Madelung potential', the potential experienced by the oxygen ion in a crystal lattice. Cohen[34] used the potential-induced breathing (PIB) modification of the MEG approach to model stishovite, a high-pressure phase of silica. In this model the oxygen interatomic potentials are parameterized in terms of the Madelung site potential: a change in the site potential induces a corresponding change in radius, or a 'breathing', of the Watson sphere. The PIB model potentials include not only pairwise-additive interactions but also many-body effects, and as a result it successfully simulates trends in the elastic constants of alkaline earth oxides, which cannot be modelled by simple two-body potentials[42]. Jackson and Gordon[33] have used a double-shell modification of the MEG method to describe the directionality of the Si-O bond, and have thus simulated the equation of state of quartz and coesite (another high-pressure phase).

From both the empirical and non-empirical methods that have been reported to date, it is obvious that a successful model of silica needs to describe both the ionic nature of the material (to model the defect, vibrational and thermodynamic properties) and the directionality of the bonding. It is unfortunate that no critical comparison has been attempted of the merits and shortcomings of the many potential models that now exist. But a more general study of potential modelling led Stoneham and Harding[43] to conclude that "at present the best empirical potentials have a far greater demonstrated accuracy than explicit solutions to the Schrödinger equation". This point seems to be echoed by Lasaga and Gibbs[19], who point to the close agreement between their quantum-mechanically derived Si-O potential and the earlier empirical potential of Parker[44]; they conclude that

TABLE 1 Properties of $MgSiO_3$ perovskite

	Calculated[46] (GPa)	Experiment[47] (GPa)
C_{11}*	531	520
C_{12}	44	114
C_{22}	531	510
C_{13}	143	118
C_{23}	166	139
C_{33}	425	437
C_{44}	237	181
C_{55}	249	202
C_{66}	136	176
K†	249	245
μ‡	192	184

* C_{ij}, elastic constants.
† K, bulk modulus.
‡ μ, shear modulus.

the "agreement supports the use of quantum-mechanical calculations as a framework within which to shape interatomic potentials".

Quantum-mechanical calculations on Mg silicates

Much recent research within the Earth sciences has aimed to determine the fundamental processes and mechanisms involved in mantle dynamics, lithospheric motion and plate tectonics. Significant advances have been made recently in our understanding of mantle phases, but future progress in resolving important geophysical problems depends upon the ability to obtain highly accurate values for the seismic and defect properties of minerals under pressure and temperature conditions appropriate to the mantle. To reproduce this environment experimentally represents a considerable challenge; as the prospect of overcoming many of the problems remains remote, the development of theoretical and computer-based atomistic models of high-density mantle-forming silicates has been given much attention. These studies should provide a more accessible way of establishing the properties of silicates at extremes of pressure and temperature, and should elucidate the microscopic or atomistic characteristics of mantle materials, which determine their bulk macroscopic and thermodynamic behaviour.

The work of Nada (to be published) and of Cohen *et al.*[45] exemplifies the current state of *ab initio* total-energy calculations on crystalline magnesium silicates. Both authors have investigated the electronic structure of high-pressure polymorphs of $MgSiO_3$; these are of particular interest as they contain octahedrally coordinated Si (Fig. 1). Nada's study of the ilmenite phase uses periodic-boundary-condition Hartree–Fock techniques. The calculations give an energy-minimized unit-cell volume and a c/a axis ratio that are within 2% of those measured experimentally. The results also indicate that octahedrally coordinated Si is significantly more ionic than tetrahedrally coordinated Si. Cohen *et al.*[45] perform total-energy calculations for cubic magnesium silicate perovskite, using the linear augmented plane-wave (LAPW) method to describe the charge density and potential surface within the unit cell. Although this approach is extremely accurate, it has the disadvantage that it is computationally very intensive—the calculation of the energy of the cubic perovskite cell (containing only five atoms) takes 10-20 c.p.u. hours on a Cray-XMP supercomputer. Although it is useful in estimating the compressibility of the hypothetical cubic magnesium silicate perovskite and in revealing the predominantly ionic nature of the bonding in such highly coordinated silicates, this computationally intensive approach is not practical at present for the general study of geophysically important phases.

Simulation of magnesium silicates

Early model simulations of silicates concentrated on reproducing the structure and elastic properties of known minerals. This requires a correct description of both the first and second derivatives of the potential-energy surface within the crystal. It was generally found, however, that either the structure could be reproduced at the expense of predicting elastic constants that were unrealistically stiff, or that reasonable elastic constants could be obtained at the expense of predicting a structure with an unrealistically low density. With the realization of the need to account for many-body interactions, however, it has become possible to obtain models that predict zero-pressure, zero-temperature densities to within 2%, and elastic constants to within 5-10%, of those determined experimentally. The ability of computer models to predict the physical properties of mantle-forming phases was illustrated recently by a PIB model calculation of the elastic properties of magnesium silicate perovskite[46]. Subsequent experimental data[47] (Table 1) are in very close agreement with the values calculated.

Excellent potential models have been developed by several workers[38-40,48-56] and have been applied to most important mantle phases. It is widely recognized[57-59], however, that if outstanding geophysical problems, such as the nature of chemical layering in the mantle, are to be resolved, very accurate constraints must be placed on the thermal-expansion coefficients and elastic constants of mantle phases. For example, a 10% uncertainty in dK/dP for magnesium silicate perovskite (K being bulk modulus and P pressure), or an uncertainty of $\pm 2.0 \times 10^{-5}$ in its thermal-expansion coefficient, has an effect on the calculated seismic behaviour equivalent to a change in the chemical composition of the lower mantle from pyrolitic to chondritic. Thus, despite being able to predict equations of state and seismic velocities with unprecedented accuracy (Fig. 2), modellers find themselves in the frustrating position of still being unable to use their calculated equations of state to discriminate between various compositional models of the mantle.

In contrast to these limitations, computer simulations are making an immediate contribution to the prediction of phase relations and of defect properties, where experimental results are poorly constrained. The experimental phase diagrams of the $(Mg,Fe)SiO_3$ and $(Mg,Fe)_2SiO_4$ systems, for example, are still subject to considerable uncertainty because of the difficulty in producing consistent pressure and temperature calibrations. The success of Price and co-workers[39,40] in reproducing the heat capacities of minerals using potentials based on the Si–O potential of Sanders *et al.*[29] means that related thermodynamic properties, such as entropy, are also predicted accurately. By accurately simulating cell volumes as well as free energies, these models can be used not only to predict the Clausius–Clapeyron slopes of phase boundaries (that is, dS/dV), but also the complete phase diagram (Fig. 3).

As the viscosity and rheological behaviour of the mantle is likely to be determined by diffusion, which is controlled in turn by the intrinsic disorder within mantle minerals, calculation of the formation and migration energies of these intrinsic defects

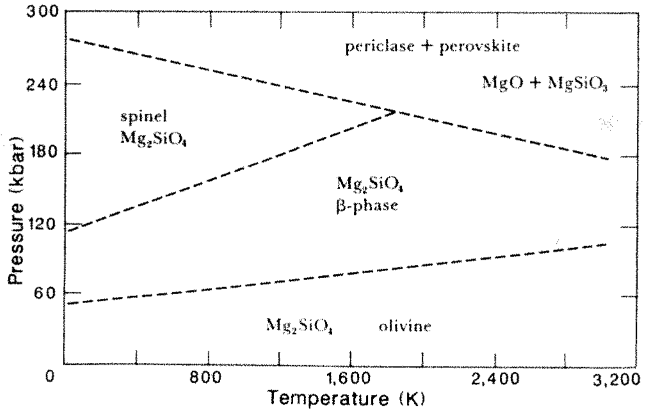

FIG. 3 The phase diagram for the magnesium orthosilicate system, calculated using free-energy simulation methods employing lattice-dynamical techniques.

is of considerable importance. The Mott–Littleton methodology has been used in association with the Si–O type potentials to model the defect behaviour of forsterite and magnesium silicate perovskite[60,61]. Calculations[60] for forsterite indicate that Mg^{2+} Frenkel pairs of defects are most favourable energetically, although a significant proportion of MgO Schottky defects is also expected. These calculations established the diffusion pathway for Mg^{2+} vacancies and interstitials and predicted an activation energy of 3.85 eV, in agreement with experimental studies[62] of Mg diffusion in olivine (which yield activation energies ranging between 3.7 and 4.5 eV). Similarly, the predicted[61] activation energy for O diffusion in magnesium silicate perovskite (0.8 eV) is almost identical to that found for O diffusion in the structurally related high-temperature superconducting oxides[63].

The movement of oxygen in the magnesium silicate perovskite lattice has also been studied recently by molecular dynamics[64,65]. The object of these studies was to investigate whether magnesium silicate perovskite is a 'superionic' conductor (a solid material showing electrical conductivity of the same magnitude as a molten salt) at mantle pressures and temperatures. Although differing in detail, the general conclusion of the two studies was that, at typical lower-mantle temperatures and pressures, magnesium silicate perovskite is indeed a superionic conductor with a completely mobile oxygen sublattice. It is predicted that the conductivity of magnesium silicate perovskite in the lower mantle will be $\sim 100\ \mathrm{S\ m^{-1}}$, in agreement with the lower-mantle conductivity inferred from the modelling of temporal variations of the geomagnetic field[66].

Remarkable progress has been made in the past five years in the development of computer simulations of magnesium silicates. But to resolve the questions concerning the detailed composition of the mantle, it will be necessary to improve both the calculation and the description of interatomic potential-energy surfaces within minerals, and to develop a better treatment of anharmonic effects in the calculation of high-temperature properties. Simulations of defects and kinetic processes are, however, already able to add to our understanding of mantle behaviour. It will hopefully be possible in the future to investigate the role of iron in determining the nature of silicate perovskites, to investigate the onset of melting of mantle phases, to

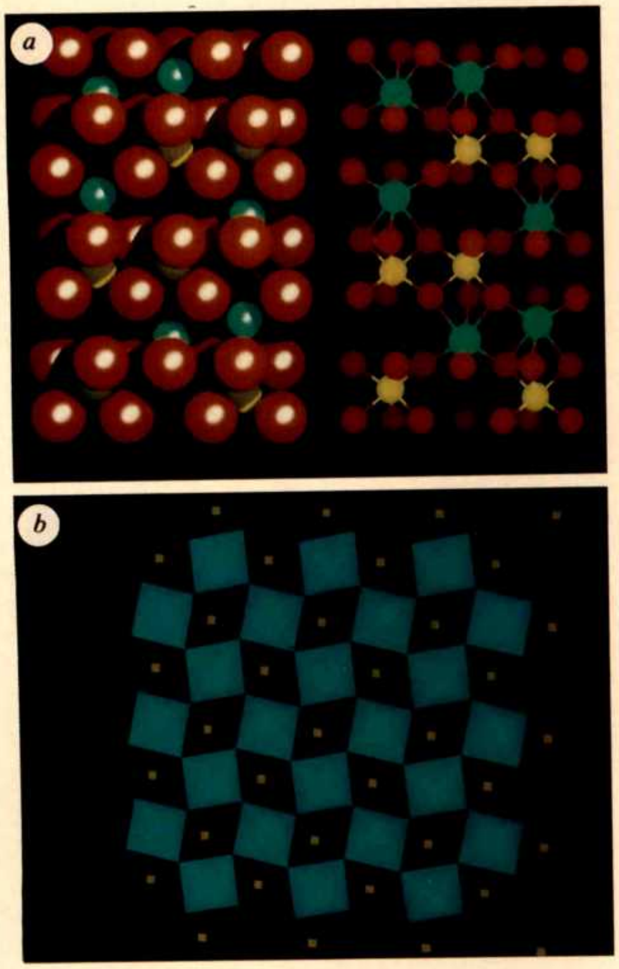

FIG. 1 The experimentally determined structures of *a*, the ilmenite phase and *b*, the perovskite phase of $MgSiO_3$. In *a* the yellow spheres are Si, the green spheres are Mg and the red spheres are O. The diagram on the left shows the relationship of the crystal structure to the close-packed oxygen sublattice. In *b* the blue polyhedra represent octahedrally coordinated Si and the yellow squares indicate Mg^{2+} ions.

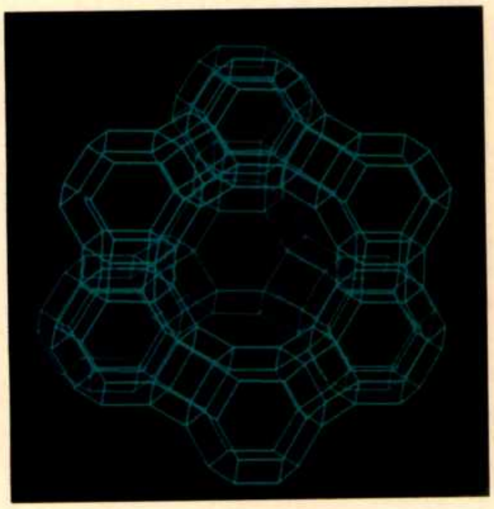

FIG. 4 The crystal structure of zeolite Y. This synthetic aluminosilicate is isostructural with the rare mineral faujasite.

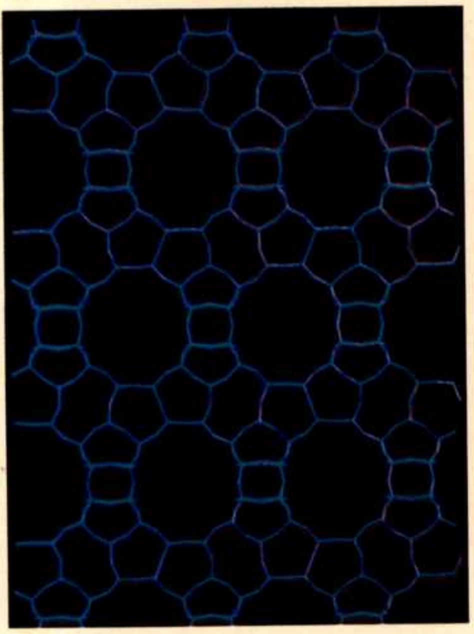

FIG. 5 The structure of 'polymorph A' of zeolite beta. The structure proposed by Newsam *et al.*[71] is shown in blue; the energy-minimized structure[72] is shown in red.

model silicate surfaces, and to extend the study of the effect of pressure on diffusion processes in silicates.

Modelling of zeolites

Zeolites are aluminosilicates (although an increasing number of almost purely siliceous materials are being prepared) with highly porous, 'framework' structures. Their current importance arises largely from their role as heterogeneous catalysts in the petrochemical industry. One of the most important examples is zeolite Y (Fig. 4), which is used extensively as a hydrocarbon-cracking catalyst. Modelling studies of these materials have concentrated on three aspects: the prediction of structures and stabilities of zeolite frameworks and of the positions of extra-framework cations; the modelling of the location and dynamics of sorbed molecules; and quantum-mechanical studies of the reaction pathways of sorbed reactive species. Here we concentrate on the first area; illustrations of the latter two can be found in refs 22 and 67.

Work over the past five years has shown that the three-body potentials derived for α-SiO_2 by Sanders *et al.*[29], when coupled with earlier empirical potentials derived for α-Al_2O_3, are able to yield excellent energy-minimized structural models for a wide range of zeolites. Structures studied include zeolite A, faujasite, silicalite and mordenite; a detailed discussion is given by Jackson and Catlow[68]. Sanders and Catlow[69] have shown that simple energy-minimization procedures can also be used to model the location of extra-framework cations in zeolite X, and Jackson *et al.*[70] have modelled the location of Ni^{2+} in Ni-mordenite and its dependence on the Al distribution in the framework. Possibly the most exciting recent application has concerned zeolite β—a structure that has long been controversial. Newsam *et al.*[71] have shown that the structure is essentially an intergrowth of two polymorphs, both of which have been modelled successfully using energy-minimization techniques. The results of these calculations[72] strongly support the structural models proposed by Newsam and Treacy[71]; the energy-minimized structure of the first polymorph (polymorph A), for example, is very close to that proposed by Newsam *et al.*[71] (Fig. 5).

Conclusion

Electronic-structure and simulation techniques are now able to undertake realistic calculations on an increasingly wide and complex range of materials. The field is developing rapidly and ambitious projects such as full electronic-structure calculations on zeolites are becoming increasingly feasible. The most exciting opportunities will involve blending simulations with electronic-structure techniques to study, for example, defects in semiconductors and the reactions of molecules in zeolites. Accurate and predictive calculations of such properties can be envisaged in the near future.

Note added in proof: A recent publication (B. W. H. van Beest, G. J. Kramer and R. A. van Santen, *Phys. Rev. Lett.* **60,** 1955 (1990)) has described a quantum mechanically derived potential for SiO_2 which successfully described the crystal properties o[illegible] the material and which has been carefully compared with the model of Sanders *et al.*[29]. □

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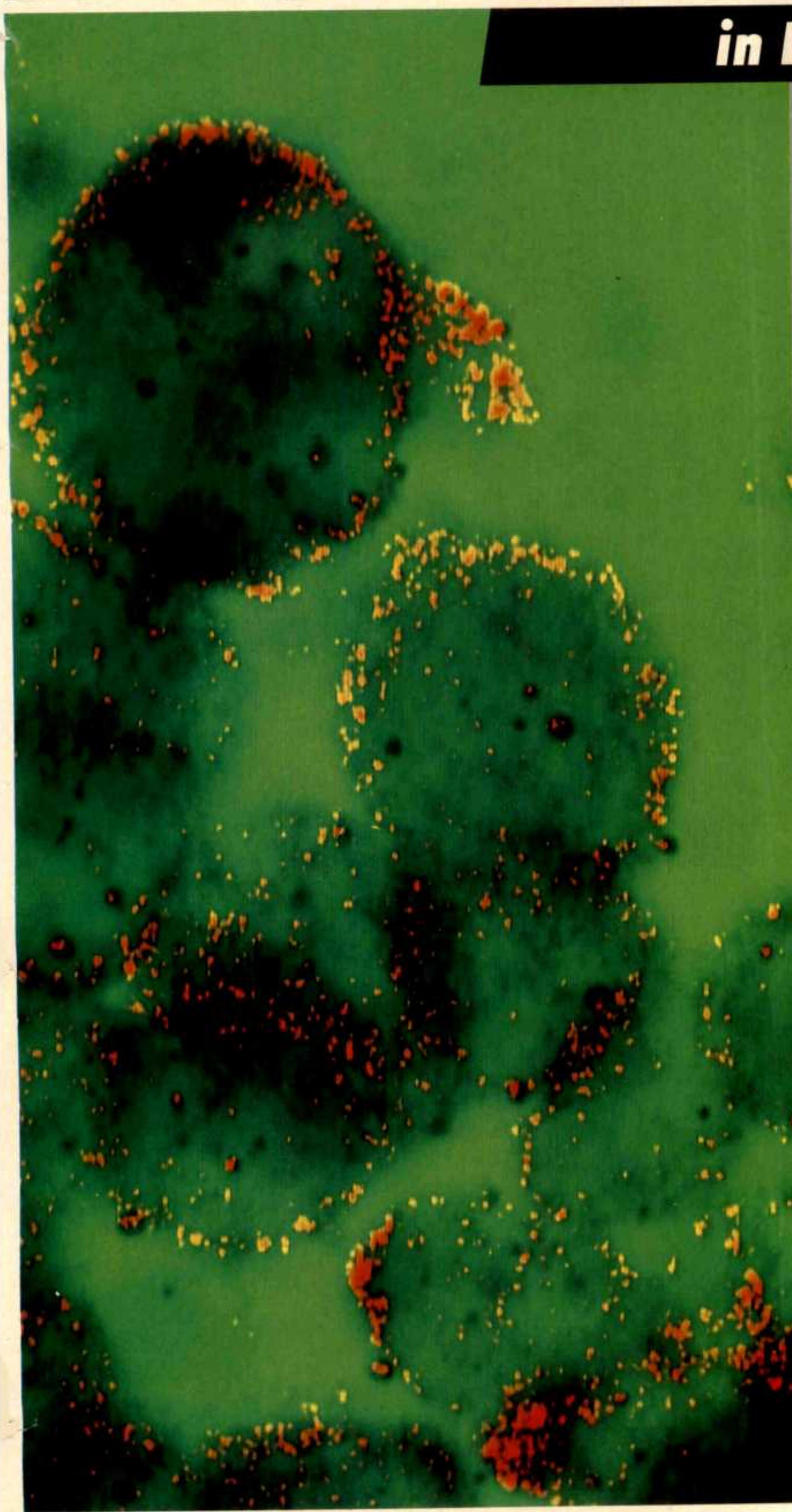

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A second class of synthetase structure revealed by X-ray analysis of *Escherichia coli* seryl-tRNA synthetase at 2.5 Å

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The three-dimensional crystal structure of seryl-transfer RNA synthetase from *Escherichia coli*, refined at 2.5 Å resolution, is described. It has an N-terminal domain that forms an antiparallel α helical coiled-coil, stretching 60 Å out into the solvent and stabilized by interhelical hydrophobic interactions and an active-site α-β domain based around a seven-stranded antiparallel β sheet. Unlike the three other known synthetase structures, the enzyme contains no classical nucleotide-binding fold, and is the first representative of a second class of aminoacyl-tRNA synthetase structures.

THE aminoacyl-tRNA synthetases are a group of enzymes, one for each amino acid, which specifically catalyse the esterification of an amino acid with a hydroxyl on the 3'-terminal adenosine of its cognate tRNA(s) (reviewed in ref. 1). The reaction requires ATP and the presence of Mg^{2+} for the formation of the aminoacyl-adenylate as an active intermediate. These enzymes thus have a crucial role in maintaining the fidelity of protein synthesis by their high degree of discrimination against incorrect tRNA aminoacylation, since messenger RNA is translated at the ribosome without reference to the amino acid attached to the tRNA. The need for such enzymes to mediate the translation of the genetic code was first suggested by Crick in 1958 as part of his adaptor hypothesis of protein assembly[2].

Much work has been undertaken to understand the molecular basis of the specificity of the aminoacyl-tRNA synthetases and the catalytic mechanism[1]. Up until 1989, only two synthetase structures had been determined by X-ray crystallography. These were the dimeric tyrosyl-tRNA synthetase from *Bacillus stearothermophilus* (YRSBS) and an active monomeric tryptic fragment of *E. coli* methionyl-tRNA synthetase (MRSEC). The structure of YRSBS is known in the presence of tyrosine[3] and of tyrosyl-adenylate[4] and that of MRSEC is known unligated and in the presence of ATP[5,40]. Despite very little sequence similarity, these two enzymes possess a structural feature in common, namely a dinucleotide-binding fold based around a five-stranded parallel β sheet, a structural motif also found in dehydrogenases and kinases[6]. Part of this structure, a 44-residue β-α-β motif is essentially identical between the two enzymes[7] and also contains in the first β-α loop, one of the few sequence motifs recognized in several aminoacyl-tRNA synthetases[8,9], His-Ile-Gly-His (the so-called HIGH region), which has important interactions with the ATP. This limited structural similarity is preserved in the most recent structure published, that of glutaminyl-tRNA synthetase from *E. coli* (QRSEC) complexed with its cognate tRNA and ATP[10]. This monomeric synthetase also contains a dinucleotide fold, similar to that found in YRSBS, and which makes many of the important contacts to the three substrates, including the ATP in the vicinity of the HIGH region.

Because of a common dinucleotide-binding domain in the three aminoacyl-tRNA synthetase structures so far determined to high resolution, it has been suggested that all synthetases may have evolved from an ancestral catalytic domain that contained this structural motif[10]. The structure of the *E. coli* seryl-tRNA synthetase (SRSEC) described here shows that the evolutionary situation is more complex. SRSEC contains no HIGH sequence motif in its primary structure and no dinucleotide-binding domain in its tertiary structure. Indeed it has no obvious structural homology at all with the other three aminoacyl-tRNA synthetases, as its principal domain, which contains the active site, is built around a seven-stranded antiparallel β sheet. We present sequence evidence that other synthetases may have structural similarities with SRSEC and show that there are at least two families of aminoacyl-tRNA synthetase structures that have almost certainly evolved from different ancestors.

SRSEC is a dimeric enzyme of 430 residues per subunit (relative molecular mass 48,414). It charges specifically the five isoaccepting $tRNA^{Ser}$ encoded by *E. coli*[11] and the recently discovered $tRNA^{SeCys}$ (ref. 12). The existence of a variety of anticodons amongst the $tRNAs^{Ser}$ is consistent with experiments demonstrating that recognition between seryl-tRNA synthetase and the $tRNAs^{Ser}$ does not involve the anticodon[13], unlike many other synthetases[14]. Sequencing, over-expression and crystallization of the enzyme have been described previously[15,16]. Here we describe the three-dimensional fold of the protein as determined by X-ray crystallography using the method of multiple isomorphous replacement (MIR).

Structure determination

Information about the high-resolution native and derivative data collections, phasing statistics and refinement are given in Table 1 and the legend to Fig. 1 (which shows representative density from the solvent flattened MIR map). Full details of the structure determination and refined structure will be published elsewhere.

The two models derived from the native and ATP+serine data (see Table 1) are essentially identical, with no evidence that ATP, serine or seryl-adenylate are actually bound to the enzyme in the case of the ATP+Ser data. But there is a small molecule, probably a detergent molecule, bound to the protein in both structures (see below).

The current atomic model refined at 2.5 Å resolution contains 410 out of 430 residues, there being three undefined loops (residues 223-229, 272-279 and 377-381). Temperature factors are generally high with an average over all protein atoms of $30.4\ \text{Å}^2$. The overall structure meets the following strong criteria of correctness: (1) crystallographic *R*-factor of 18.8% for reflections between 7 and 2.5 Å $(I > 2\sigma)$; (2) good geometry (for example, root mean square (r.m.s.) deviation in bond lengths of 0.018 Å); (3) the main chain of the molecule can be rebuilt using fragments from the database of well refined structures to within an r.m.s. accuracy of 0.3 Å for all main-chain atomic positions (using the program MAXSPROUT; L. Holm and C. Sander, *J. molec. Biol.*), except that no fragment fits well the peptide centred at Ala 394. This is a peculiar three-glycine peptide (Gly-Ser-Gly-Leu-Ala 394-Val-Gly) between β strand A6 and helix H12, with an unusual *cis*-peptide between Gly 392 and Leu 393; (4) all but 10 of the ϕ/ψ angles are within the allowed zones, nine are close to the allowed zones and the exceptional case (Trp 363, $\phi = 57°$, $\psi = -140°$) seems to fit well the electron density in an omit map and (5) program DSSP[17] reports that there are on average 74 main-chain hydrogen bonds per 100 residues, which is above average for proteins in the Brookhaven protein databank (C. Sander, personal communication) and indicates a well stabilized protein structure. Cα positions will be deposited in the Brookhaven protein databank.

Description of the structure

Seryl-tRNA synthetase consists of two domains. The first 100 N-terminal residues form an all α domain containing a remarkable solvent exposed arm consisting of an antiparallel coiled-coil of α helices and about 60 Å long. The rest of the molecule forms a large globular α–β domain built around an eight-stranded β sheet (seven antiparallel strands and one short parallel strand at one edge, forming part of the dimer interface). Overall the protein contains about 40% α helix and 20% β sheet (in the globular domain alone, residues 101–430, the amounts are 26% and 24%, respectively). Figure 2*a* shows the Cα backbone of the monomer and Fig. 2*b* shows the dimer viewed along the two-fold axis. Figure 3 shows a schematic diagram of the folding topology with the main secondary structural features indicated.

The helical arm. The helical arm is composed of two very long antiparallel helices, H3 of 10 turns (residues 27–64) and H4 of nine turns (residues 69–100) joined by a short segment of extended chain (residues 66–68). The arm makes an angle of about 55° to the twofold axis; in the dimer, the two arms are nearly perpendicular with a distance of ~110 Å between their tips (see Fig. 2*b*). The arm structure completely lacks aromatic residues and 40% of the residues are charged. Helix H3 has an overall positive charge (nine positive, five negative), whereas helix H4 has an overall negative charge (four positive, nine negative). Including the loop, there is an overall net charge of −2. Whereas helix H3 takes part in important crystal contacts, the outer surface of helix H4 is exclusively in contact with solvent, and consequently its temperature factors are higher.

The two helices in SRSEC form the longest antiparallel pair so far reported in the literature and curve around each other with a slight left-handed twist. They do not form a regular coiled-coil as found in fibrous proteins such as tropomyosin, myosin and fibrinogen[18], in which the individual helices have a left-handed supercoil and form a parallel pair with a left-handed twist of pitch about 150 Å. The individual SRSEC helices have a right-handed supercoil and form an antiparallel pair with a left-handed twist. This geometry cannot extend indefinitely, but can occur for a limited length (provided the supercoil pitch is large) while maintaining close interhelical contact. Indeed the (straight) axes of the major helices in the individual coiled-coils in SRSEC make an angle of 130° and the interaxis separation is 8.8 Å in the middle, diverging to about 10.5 Å at the ends.

Apart from the normal α-helical hydrogen bonds, the helical arm is stabilized by interactions of three types: (1) a core of hydrophobic interactions, (2) interhelical salt bridges and hydrogen bonds and (3) intrahelical salt bridges and hydrogen bonds. Interactions of type (2) and (3) will be described in more detail elsewhere, as well as the crystal contacts that the arm makes. Here we discuss only the main hydrophobic interactions that occur in the arm (Fig. 4*a*).

The legend to Fig. 4*a* shows the sequence of the two helices arranged in heptads as normally used in discussions of coiled-coils[18]. Helix H4 has heptads following rather closely the average composition in α fibrous proteins[18] with exclusively hydrophobic residues in positions a and d. Helix H3 has a less characteristic sequence, in particular in position a, normally hydrophobic in a classical coiled-coil, there are two arginines (having long aliphatic chains) and a threonine. The interhelical hydrophobic interactions involve all residues in position a and d from both helices and result in a continuous hydrophobic

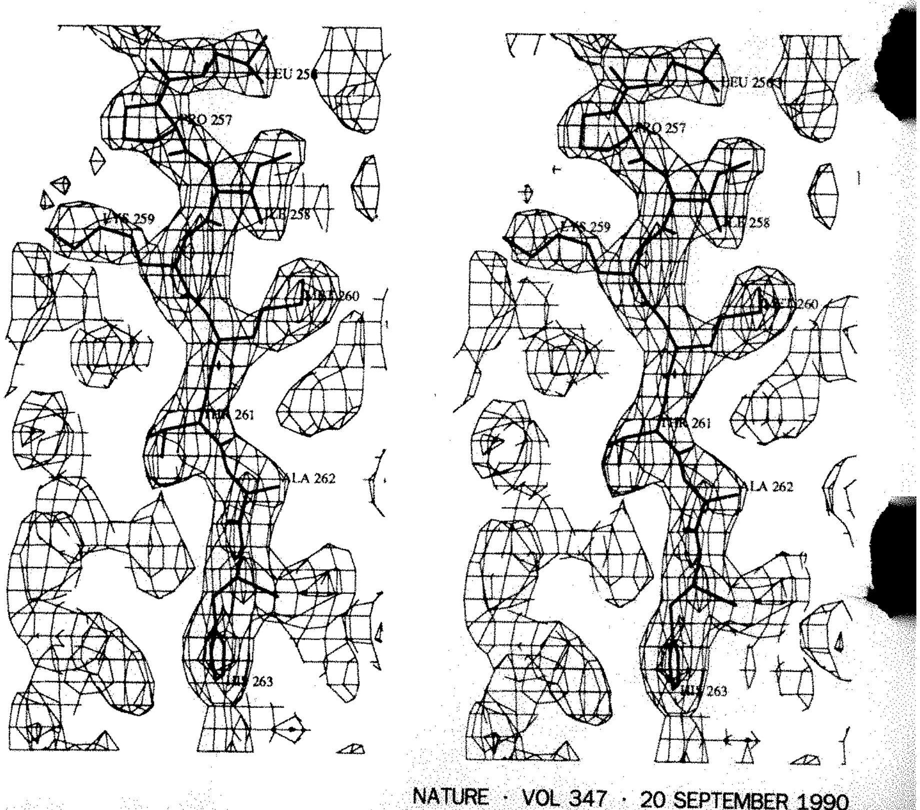

FIG. 1 Stereo view of the solvent-flattened MIR electron density at the beginning of β strand A7 including *cis*-proline 257, with the current atomic model superposed. This density was obtained, using the ATP+Ser F_{obs}, after repeated cycles of solvent flattening combined with gradual phase extension to 2.5 Å starting with the 2.8 Å MIR map. The contour level is 0.85σ.

METHODS. After initial data reduction, all crystallographic calculations were performed with the CCP4 program package (Daresbury Laboratory). MIR phases to 2.8 Å were calculated using three heavy-atom derivatives, ethylmercury chloride (two sites), dysprosium chloride (one site) and uranyl-acetate (a compact cluster of nine sites) and the resultant electron density map was improved by solvent flattening[37]. Anomalous differences were included for the dysprosium and uranyl derivatives. An initial model was built using the BONES and fragment fitting options of FRODO[38] and subsequently refined using the GROMOS molecular dynamics refinement package[39]. The current R-factor is 18.6% for the native data (all data 6–2.8 Å, no solvent, individual temperature factors refined) and 18.8% for the ATP+Ser data (all data with $I > 2\sigma$ between 7–2.5 Å, individual B-factors refined, 95 solvent molecules, R=20.2% for all measured data).

TABLE 1 Statistics of data collection and data analysis

Derivative	Native*	$DyCl_3$†	UO_2Ac‡	EtHgCl§	ATP + serine‖
Detector	Xentronics¶	Film *	Xentronics	Xentronics	Imageplate**
Wavelength (Å)	1.54	1.43	1.54	1.54	0.96
Total No. reflections/No. crystals	38,631/2	49,034/12	30,128/1	31,057/2	72,569/2
Unique reflections (% complete)	18,573 (88%)	17,918 (90%)	18,269 (91%)	13,953 (77%)	28,046 (98%)
Resolution limit (Å)	2.75	2.8	2.8	2.9	2.5
R_{merge}	0.059	0.059	0.068	0.067	0.059
Number of heavy atom sites		1	9	2	—
Isomorphous difference‡‡		0.11	0.20	0.11	0.11
R_{Cullis}§§		0.69	0.41	0.67	
Anomalous scattering used?		Yes	Yes	No	
Phasing power¶¶: all reflections to 2.8 Å (3.1–2.8 Å shell)		1.43 (1.09)	5.79 (4.06)	1.50 (0.94)	
Mean figure of merit for 16,054 reflections to 2.8 Å (3.1–2.8 Å shell)	0.69 (0.50)				

* SRSEC crystallizes in monoclinic space-group I2 (the body-centred equivalent of standard space-group C2) with unit cell parameters of $a = 128.1$ Å, $b = 90.7$ Å, $c = 69.8$ Å, $\beta = 91.1°$. Crystals are routinely obtained by equilibration of a 1% protein solution in 67 mM Tris, 50 mM HCl, 10 mM $MgCl_2$, 1 mM DTE pH 7.6, 1 M ammonium sulphate and 1% of a particular batch of octyl-glucoside (practical grade, Pfanstiehl) against 52% saturated ammonium sulphate at room temperature. A 5 min centrifugation in a Beckman Airfuge at maximum speed is necessary before equilibration, to remove excess crystal nuclei. The detergent sample has been analysed by gas chromatography and is an impure mixture of ~40 components; the active ingredient has not been identified. After growth the crystals can be transferred to mother liquor, which does not contain detergent, whereupon the *a* cell axis increases to 129.7 Å and the crystals are less stable. These observations explain the variability in cell dimensions described previously[16]. In the work described here, detergent was always present. The asymmetric unit is the monomer, implying a solvent content of about 65%.

† Soaked for 4 days in 10 mM dysprosium chloride.

‡ Soaked for 3 days in 0.8 mM uranyl acetate at pH6.8.

§ Soaked for 4 h in 0.01 mM ethylmercury chloride.

‖ Soaked for 2 days in 4 mM ATP +4 mM L-serine.

¶ Xentronics data were collected with 0.2° frames (120 s) and arbitary crystal orientation and integrated with the XENGEN package.

# Film data were collected on synchrotron line D41 at LURE using a wavelength of 1.43 Å to enhance the anomalous signal from the dysprosium (L_{II} edge is at 1.444 Å). Oscillation photographs (2°) were recorded with rotation about the unique twofold axis to obtain accurate measurements of Bijovet differences with a crystal-film distance of 90 mm and exposure times of 11–16 min. Films (three per pack) were scanned with pixel size 50 μm using an Optronics rotating drum scanner and processed using the Imperial College package including profile fitting. Only full reflections were used.

** Image-plate data were collected at the EMBL Hamburg Outstation on beamline X11 using the EMBL camera-scanner system. Images were recorded with rotation about the crystallographic a^*-axis in two passes: first a high resolution pass with oscillation range 2°, crystal-plate distance 200 mm and exposure times of 7–14 s and then a low-resolution pass with oscillation range 3°, crystal-plate distance 288 mm and an attenuated beam. A second crystal was used to complete the blind region. Reflections were integrated with the Imperial College package without profile fitting (adapted for the image-plate by the EMBL Hamburg Outstation). Full and summed partial reflections were used.

†† $R_{merge} = \sum\sum |I_{j(h)} - \langle I(h)\rangle| / \sum\sum I_j(h)$, summed over all measured reflections.

‡‡ Isomorphous difference $= \sum |F_{nat} - F_{deriv}| / \sum F_{nat}$.

§§ $R_{Cullis} = \sum |FPH_{calc} - FPH_{obs}| / \sum |FPH_{obs} - FP|$, where FPH and FP are the derivative and native structure factor amplitudes and the sum is over centric reflections only.

¶¶ Phasing power $= f_h/E$ where f_h is the r.m.s. heavy atom structure factor amplitude and E is the r.m.s. lack of closure error. Phasing statistics are as given by the final run of the CCP4 program PHARE before calculation of the first MIR electron density map. Undoubtedly the results are biased by the strong phasing power of the uranium derivative. Indeed, this derivative in itself would have been sufficient to solve the structure, although the other derivatives were necessary to place the cluster of uranium atoms.

core. However, the relative longitudinal stagger of the two helices, combined with the fact that in an α helix the sidechains tend to point back to the N terminus, mean that the most direct interactions are between residues in position a in one helix with position d in the second helix (Fig. 4*b*). Of particular interest are a number of interhelical leucine-(iso)leucine pairs. For instance, the heptad repeat of three leucines (Leu 33, Leu 40 and Leu 47) in position d in the first helix interact, respectively, with a similar repeat of an isoleucine and two leucines (Ile 96, Leu 89, Leu 82) in position a in the second helix. There are two other leucine-isoleucine interhelical pairs (Leu 30/Ile 99 and Ile 58/Leu 71), as well as two occurrences of interhelical leucine-arginine interactions. In these interacting pairs, the side chains point towards each other (Fig. 4*b*). To avoid unfavourable contacts in the middle, they are systematically turned slightly outwards in opposing directions and lie side by side (Fig. 4*a*). This arrangement of interhelical interacting side chains is most appropriately called a 'leucine ladder'.

The globular domain. The globular domain is an α–β domain comprising residues 101–430 built around a seven-stranded antiparallel β sheet (sheet A in Fig. 3), with a short eighth parallel strand at the dimer interface (see below). There are three other short antiparallel β sheets (B, C and D in Fig. 3) in this domain as well as five short helices and two long α helices. One of the long helices (H7, residues 167–186) is perpendicular to the twofold axis at the top of the molecule and interacts with the symmetry-related helix as part of the dimer interface. The second long helix (H10, residues 301–319) forms a right-handed cross-over connection between β strands A6 and A2 and packs on the outside (top) surface of the β sheet. The three poorly defined loops are in this domain (residues 223–229, 272–279 and 377–381). Out of 19 prolines in the enzyme, there are two *cis*-prolines (Pro 211 and Pro 257), both preceded by leucines, in accordance with the marked preference in this position for hydrophobic residues unbranched at Cβ (ref. 19).

Dimer interface. The most important inter-monomer interactions occur in the long insertion of 132 residues between β-strands A1 and A7. The antiparallel helices H7 (residues 169–186) and H7* (residues 169*–186*, where * indicates the second monomer) interact across the twofold axis by means of complementary charged and polar side chains. The other principal features of the dimer interface involve two short inter-monomer antiparallel β sheets. The first is a continuation of the main eight-stranded β sheet with strands A9* and A10* being hydrogen bonded to a short stretch (residues 260–261) of strand A8 (Fig. 2*b*). The second is a symmetrical six-stranded antiparallel sheet (visible close to the twofold axis in Fig. 2*b*) comprising strands B1, B2 and B3, which interact with their

TABLE 2 Homology of seryl-, threonyl- and prolyl-tRNA synthetases in the active site

						← βA7 →													← disordered →														← βA6 —			
SRSEC	254	D	D	**L**	**P**	I	K	M	T	A	H	T	P	C	F	**R**	S	**E**	A	G	S	Y	G	R	D	T	R	**G**	L	I	**R**	M	H	Q	**F**	D
SRSSC	265	E	Q	**L**	**P**	I	H	Y	V	G	Y	S	S	C	F	**R**	R	**E**	A	G	S	H	G	K	E	A	W	**G**	V	F	**R**	V	H	A	**F**	E
PRSEC	126	K	Q	**L**	**P**	L	N	F	Y	Q	I	Q	T	K	F	**R**	D	**E**	V	R	P	R	F					**G**	V	M	**R**	S	R	E	**F**	L
TRSEC	349	R	D	**L**	**P**	L	R	M	A	E	F	G	S	C	H	**R**	N	**E**	P	S	G	S	L	H				**G**	L	M	**R**	V	R	G	**F**	T
TRSSCM	148	N	E	**L**	**P**	L	R	F	S	D	F	S	P	L	H	**R**	N	**E**	A	S	G	A	L	S				**G**	L	T	**R**	L	R	K	**F**	H
TRSSC	435	R	E	**L**	**P**	W	R	V	A	D	F	G	V	I	H	**R**	N	**E**	F	S	G	A	L	S				**G**	L	T	**R**	V	R	R	**F**	Q
TRSBS2	351	R	D	**L**	**P**	I	R	M	A	E	F	G	Q	V	H	**R**	H	**E**	Y	S	G	A	L	N				**G**	M	L	**R**	V	R	T	**F**	C
TRSBSV	353	R	E	**L**	**P**	I	R	I	A	E	L	G	T	M	H	**R**	Y	**E**	M	S	G	A	L	S				**G**	L	Q	**R**	V	R	G	**M**	T
			+	*	*	+	+	+			+			+	+	*		*			+							*	+		*	+	+		+	

Homology of the sequences of seryl-tRNA synthetases from *E. coli* (SRSEC) and *Saccharomyces cerevisiae* (SRSSC), prolyl-tRNA synthetase from *E. coli* (PRSEC[25]) and threonyl-tRNA synthetases from *E. coli* (TRSEC), *S. cerevisiae* both cytoplasmic (TRSSC) and mitochondrial (TRSSCM), and two enzymes from *Bacillus subtilis* (TRSBS2 and TRSBSV)[36]. * Conserved residues, shown in bold type; +, conservative changes. The secondary structure in SRSEC is indicated.

symmetry-related counterparts (B3 and B3* being antiparallel at the interface).

Putative active site. There are neither crystallographic nor biochemical data on the sites of binding of any of the substrates (ATP, serine, tRNA) to SRSEC. But examination of the structure reveals a putative active site in the globular domain. Whereas one side of the β sheet is overlaid by the long helix H10 (residues 301–319) forming a convex surface, the other side forms the floor to a deep depression, rimmed by loops and visible in Fig. 5. Of particular note is the loop formed by residues 269–285, part of which is not clearly visible in the electron density map (residues 272–279). This loop contains sequence motifs conserved among certain other synthetases, suggesting its functional importance (see discussion). We imagine it to form a flexible lid over the active site region, which may be stabilized on binding of substrates. There are several basic residues in the putative active site (for example, Arg 268, 283, 397 and Lys 289), reminiscent of MRSEC, where there are seven lysines and one arginine in the active site[40]. The cluster of nine uranium atoms, bound on soaking the crystals in uranyl acetate, and the dysprosium atom are in the putative active site.

There is strong residual positive electron density in the region of the active site of SRSEC, which is present in native crystals in mother liquor containing detergent, native crystals transferred to mother liquor without detergent and in crystals soaked with ATP and ATP+serine. The density is in the form of an elongated 'tadpole' about 13 Å long and because of its universal presence it is probably a very tightly bound detergent molecule, arising from the crystallization medium (see legend to Table 1). The position of this molecule is under the loop 269–285 (in particular under the strand Gly 280-Leu-Ile-Arg), with its tail buried in a hydrophobic pocket and its head in a more hydrophilic environment. The detergent molecule may be of the octyl-glucoside type.

In the crystal, important intermolecular contacts occur in the region of the active site. The end of the antiparallel arm from a symmetry-related dimer is inserted into the putative active site ('foot in mouth'). Within the dimer itself, the acidic extremity of the first disordered loop from one monomer (residues Glu 224-Glu-Glu-Ala-Asp 228) probably crosses over into the active site region of the other monomer. From these observations we suggest that because of the tight binding of the detergent molecule and the crystal packing constraints, the active site no longer has the conformational flexibility to allow true substrate binding in the crystal. Co-crystallization of SRSEC with its small molecule substrates (ATP and serine), in the presence of detergent, again shows no indication of substrate binding; in the absence of detergent, no crystals are obtained. This suggests that crystals only occur as a result of the, presumably small, conformational or surface effects of the detergent binding, which then precludes further substrate binding. In solution, the presence of detergent does not interfere with enzyme activity in the tRNA charging reaction. A low-resolution neutron diffraction study is being undertaken to determine whether there are other detergent molecules bound less specifically, using methods previously applied to the detergent-containing crystals of the photosynthetic reaction centre[20].

Discussion

The structure described above shows that SRSEC is the first example of an aminoacyl-tRNA synthetase that does not possess a dinucleotide-binding fold, thus contradicting a recent proposition that all synthetases had a common precursor containing this fold[10]. Whereas in the dinucleotide-binding fold the active site is formed by the loops at one edge of a parallel β sheet, in SRSEC the putative active site is formed on top of the central part of an antiparallel β sheet. The radically different nature of these active site domains may imply important differences in the mode of binding of the ATP, the 3′ end of the tRNA and even the catalytic mechanism. Thus there are at least two distinct families of synthetases, which may have evolved from different ancestral enzymes. Further evidence for the partition of the aminoacyl-tRNA synthetases into families comes from the existence of certain conserved motifs in known synthetase primary sequences. One group (including the enzymes for arginine, glutamine, glutamic acid, isoleucine, leucine, methionine, tryptophan, tyrosine and valine)[9] possess the HIGH motif, which is generally very close to the N terminus of the protein and which has been demonstrated in the three-dimensional structures of YRSBS, QRSEC and MRSEC to be associated with a conserved structural feature (part of the dinucleotide fold). They also all possess a Lys-Met-Ser-Lys-Ser (KMSKS) motif implicated in the binding of the 3′ end of tRNA[21]. A second group (including the enzymes for lysine, aspartic acid, asparagine, phenylalanine, glycine, alanine and histidine) lacks the HIGH motif but contains a conserved Gly-Ile/Leu-Glu/Asp-Arg (GI/LE/DR) motif[22–24]. A survey of the ~40 known aminoacyl-tRNA synthetase sequences confirms the partition of the synthetases into two groups on the basis of these conserved motifs (P. Argus *et al.*, unpublished results).

There is a region of homology between the known sequences of seryl-, threonyl- and prolyl[25]-tRNA synthetases (Table 2). Using the SRSEC structure we can assign this region to β strand A7 and the partly disordered loop (268–285) connecting β strands A7 and A6 (see Table 2 and Fig. 5). There are strictly conserved residues at the beginning of strand A7 and in the connecting loop between strands A7 and A6, which seems to form a flap over the putative active site. Pro 257 in SRSEC is preceded by a *cis*-peptide and the strict conservation of the Leu-Pro in the two other synthetases suggests that this particular structural detail is also conserved. There is also a motif Phe/His-Arg-X-Glu (F/HRXE) which is found in certain synthetases of the second class[23] and a motif Gly-Leu-X-Arg (GLXR) where X is hydrophobic or nonpolar; both strictly conserved arginines are in the putative active site in SRSEC.

The idea that the aminoacyl-tRNA synthetases fall into two distinct classes has been further elaborated recently with the

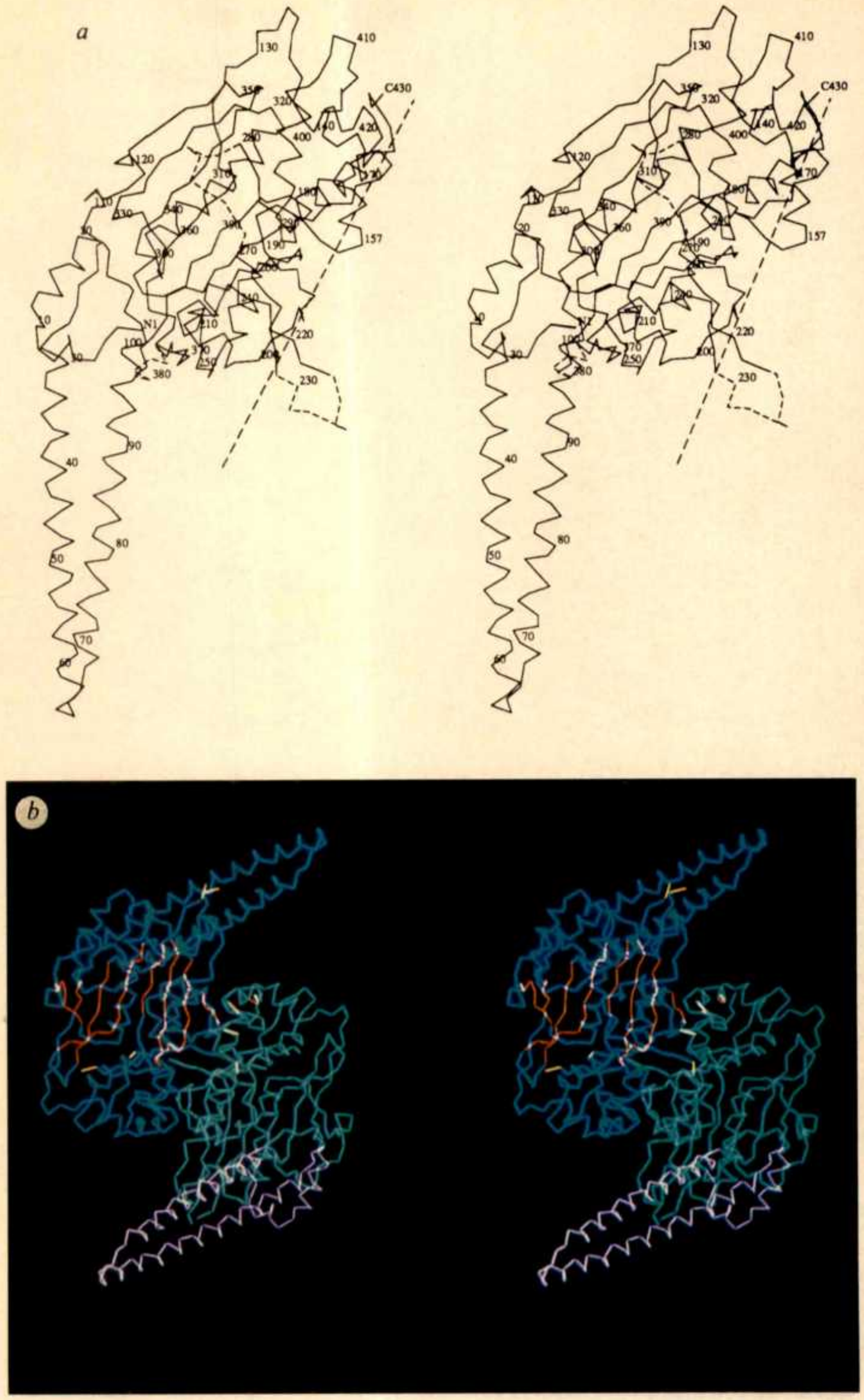

FIG. 2 *a*, Stereo view of Cα backbone of one monomer of seryl-tRNA synthetase including the approximate position of the three ill-defined loops (dotted). The dimer twofold axis is shown (dashed). *b*, Stereo view of Cα backbone of the dimer of seryl-tRNA synthetase viewed along the twofold axis. In one monomer the helical arm domain (mauve) and the globular domain (turqoise) are shown and in the other monomer the three chain breaks are shown (yellow). The main β sheet, with eight strands in one monomer and two strands in the other, is shown (red).

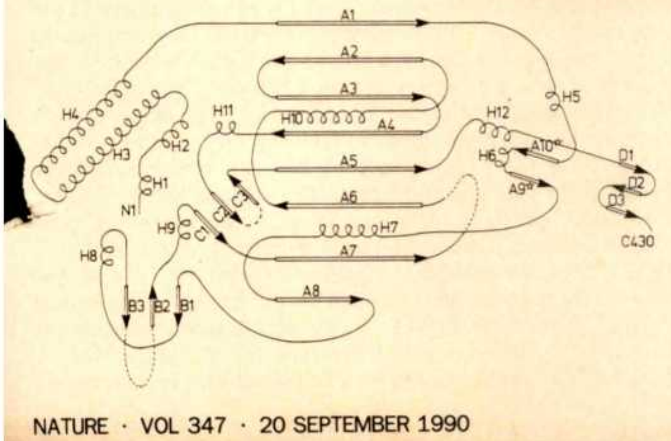

FIG. 3 Schematic diagram of main-chain fold of one monomer of seryl-tRNA synthetase. Principal secondary structural elements as determined by the algorithm of Kabsch and Sander[17] are shown. Helices: H1 (residues 4–9), H2 (11–19), H3 (27–64), H4 (69–100), H5 (139–145), H6 (151–157), H7 (167–186), H8 (201–207), H9 (239–243), H10 (301–319), H11 (365–370), H12 (395–406). β strands: A1 (121–126), A2 (323–327), A3 (339–347), A4 (352–363), A5 (386–392), A6 (286–296), A7 (258–267), A8 (191–194), A9* (164–166), A10* (149–150), B1 (198–200), B2 (232–234), B3 (219–221), C1 (250–252), C2 (373–375), C3 (382–384), D1 (407–408), D2 (413–414), D3 (428–429). Relative directions of the β strands are indicated. Drawing by J. Sedita.

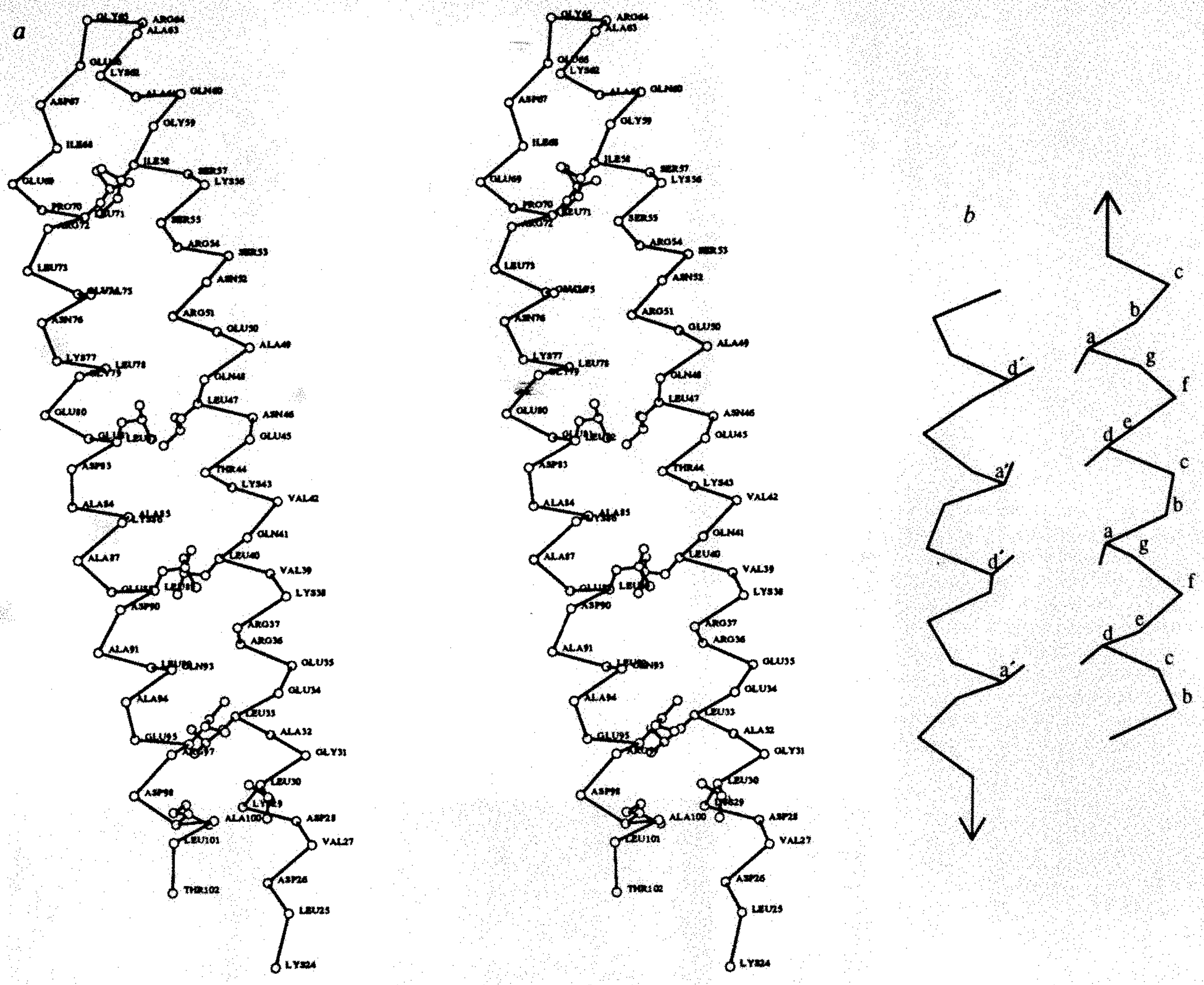

FIG. 4 *a*, Stereo figure of the Cα positions in the helical arm showing interhelical leucine–(iso)leucine bridges. The sequence of the helices H3 and H4 arranged in heptads is as follows:

a	b	c	d	e	f	g
HELIX 3						
				Val	Asp	Lys
Leu 30	Gly	Ala	Leu 33	Glu	Glu	Arg
Arg 37	Lys	Val	Leu 40	Gln	Val	Lys
Thr 44	Glu	Asn	Leu 47	Gln	Ala	Glu
Arg 51	Asn	Ser	Arg 54	Ser	Lys	Ser
Ile 58	Gly	Gln	Ala 61	Lys	Ala	Arg
HELIX 4						
	Glu	Pro	Leu 71	Arg	Leu	Glu
Val 75	Asn	Lys	Leu 78	Gly	Glu	Glu
Leu 82	Asp	Ala	Ala 85	Lys	Ala	Glu
Leu 89	Asp	Ala	Leu 92	Gln	Ala	Glu
Ile 96	Arg	Asp	Ile 99	Ala		

b, Schematic diagram showing the origin of the preferential interactions between heptad positions a and d in the first helix with, respectively, d′ and a′ in the second helix because of the particular longitudinal stagger of the antiparallel helices and the backward pointing direction of the Cβ atoms (see also *a*).

sequencing of the prolyl-tRNA synthetase from *E. coli* and the identification of three sequence motifs common to the second class of synthetases; the seryl-, prolyl- and threonyl- enzymes form a closely related subgroup within the second class[25]. The degree of structural similarity between class 2 enzymes will soon become evident with the completion of the crystal structure determination of the aspartyl-tRNA synthetase: tRNA^{Asp} complex from yeast[26]. The existence of different classes of synthetases has implications for the evolution of these enzymes and for the development of the genetic code. With a more limited database Wetzel[27] considered possible evolutionary relationships between the synthetases and noted the correlation of certain biochemical properties of synthetases (such as 2′ or 3′ ester formation and nature of the discriminator base) with codon family of the amino acid. The close sequence relationship of the enzymes for amino acids with XUX codons (notably isoleucine, leucine, valine and methionine; all class 1 synthetases) has been established[28] and for some members of the XAX codon group[23]. Three members of the XCX codon group (serine, threonine and proline) also form a closely related subgroup of class 2 synthetases (Table 2 and ref. 25).

The biological role of the helical arm of SRSEC is not yet experimentally demonstrated but the simplest hypothesis is that it is an extended strut to support the anticodon stem of the tRNA. In this role it would be analogous to the helical domain of MRSEC, which is composed of a bundle of more or less oriented helices[40]. One important difference, however is that in MRSEC the only specific recognition between tRNA and synthetase is through the anticodon and the extremity of the helical domain[40], whereas in SRSEC the anticodon is unimportant for recognition[13]. This may help to explain the ‘minimalist’ nature of the SRSEC supporting arm. The helical arm is structurally

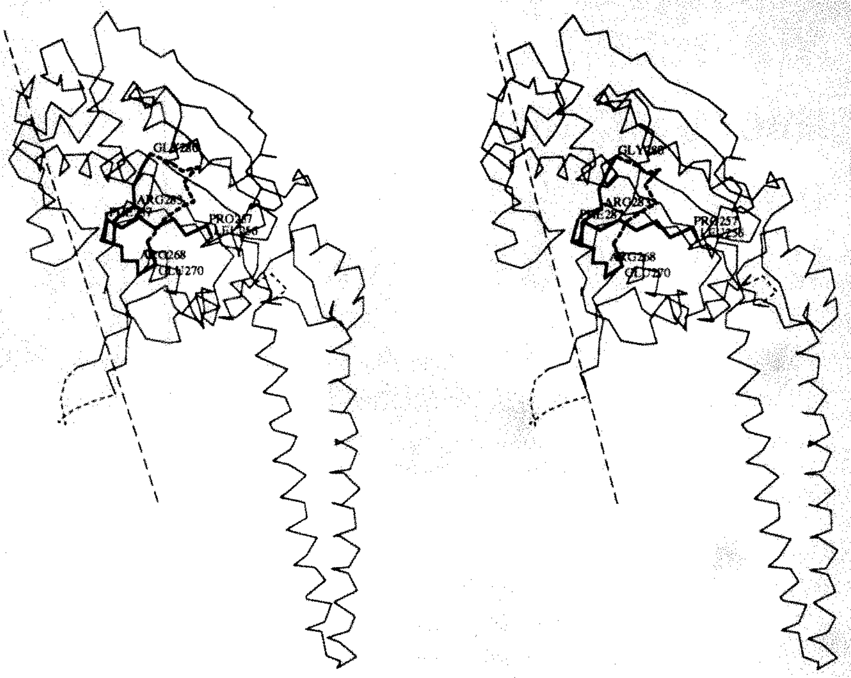

FIG. 5 Stereo view into the putative active site showing the region of homology between seryl-prolyl- and threonyl-tRNA synthetases (bold). The strictly conserved residues are marked (see Table 2). Disordered loops are dotted.

unusual and allows a detailed view of what is required to stabilize long helices in solution. This is of interest in relation to the class of DNA-binding proteins characterized by a sequence pattern comprising 3-5 leucine heptad repeats adjacent to a highly basic region[29,30]. The repeated leucine motif (misnamed 'leucine zipper') has been demonstrated in some cases to mediate homo- or heterodimerization of domains through the formation of a parallel coiled-coil[31,32]. Although SRSEC is not a member of this class of protein, the kind of interactions observed in the SRSEC arm may also be found in parallel coiled-coil structures with leucine heptad repeats.

Another RNA binding protein, ROP, involved in the regulation of replication of ColE1 plasmids, also contains a coiled-coil of antiparallel helices twisted into a left-handed supercoil[33]; the active form is a dimeric four-helix bundle. ROP binds weakly but specifically to the stem structure of the two RNA molecules involved in the regulatory mechanism and it has been proposed that simultaneous binding of the two RNA molecules to the ROP dimer mediates their hybridization[34]. It is possible therefore that the antiparallel helical motif is found in other double-stranded RNA-binding proteins. Conversely, SRSEC itself may be involved in interactions with other RNA molecules. In this connection we note that threonyl-tRNA synthetase in *E. coli* negatively regulates its own translation by binding to an operator on its mRNA, part of which is structurally similar to the anticodon stem of tRNA^{Thr}, just upstream from the coding region[35].

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Molecular cloning of the microtubule-associated mechanochemical enzyme dynamin reveals homology with a new family of GTP-binding proteins

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A complementary DNA encoding the D100 polypeptide of rat brain dynamin—a force-producing, microtubule-activated nucleotide triphosphatase—has been cloned and sequenced. The predicted amino acid sequence includes a guanine nucleotide-binding domain that is homologous with those of a family of antiviral factors, inducible by interferon and known as Mx proteins, and with the product of the essential yeast vacuolar protein sorting gene *VPS1*. These relationships imply the existence of a new family of GTPases with physiological roles that may include microtubule-based motility and protein sorting.

THE cytoplasmic microtubules of mammalian brain tissue are composed of tubulin and a diverse set of microtubule-associated proteins (MAPs). Of these, three proteins are mechanochemical nucleotide triphosphatases: kinesin[1,2], dynein[3,4] and dynamin[5]. Kinesin[6] and dynein[7] produce force in opposite directions along microtubules. They are therefore thought to be involved in anterograde and retrograde axonal transport, respectively, as well as other forms of intracellular movement (reviewed in ref. 8). Only the heavy chain of kinesin (relative molecular mass 110,000–135,000; M_r 110–135K), and several kinesin-like proteins, have been characterized at the level of primary structure[9-14]. This has revealed the existence of a common force-producing 'head' domain of ~350 amino acids, although the location of this domain within the linear sequence of the polypeptide varies[9-15].

Dynamin has a microtubule-activated ATPase activity and binds microtubules in a nucleotide-dependent manner, like kinesin and dynein, although many of its properties are distinct from those of the other two proteins[5]. The principal polypeptide of dynamin is a species of M_r ~ 100K (here termed D100) which is a prominent component of mammalian brain microtubules prepared in the absence of added nucleotides. Preparations of D100 crosslink microtubules into regular bundles, which dissociate and elongate in an ATP-dependent manner[5]. The ATPase and sliding activities required an unidentified co-factor in addition to D100[5].

To obtain further insight into the mechanism of action of dynamin, and to examine its potential relationship to other proteins of interest, we have obtained a cDNA clone encoding the entire D100 polypeptide. We report here the predicted amino-acid sequence of D100 and identify a novel family of D100 homologues.

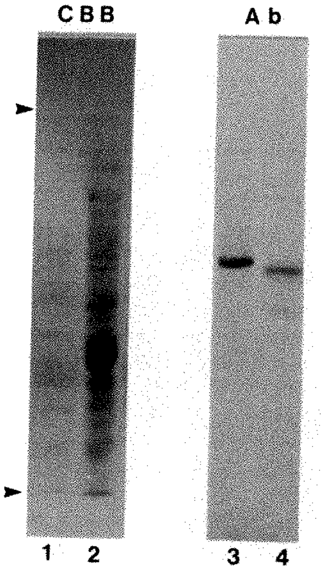

FIG. 1 Immunoblotting of dynamin-1 fusion protein and rat brain extract with affinity-purified anti-D100 antibody. Lanes 1, 3, Isopropyl thiogalactoside-induced plaques from clone λZAP-dynamin-1 (three plaques per lane). Lanes 2, 4, Adult rat brain extract (~5 μg per lane). Proteins were stained with Coomassie brilliant blue (CBB, lanes 1, 2) or affinity-purified anti-D100 (Ab, lanes 3, 4). The top of the blot and the dye front are marked by arrowheads.

METHODS. Electroblotting: A 9% gel[48] was immunoblotted[49] onto Immobilon PVDF (Millipore, Inc.) and stained with 0.1% CBB in 45% isopropanol, 10% acetic acid, or incubated with autoimmune rabbit antibody, affinity-purified[50] from an immunoblotted band of bovine brain D100[5]. Reaction products were visualized with alkaline-phosphatase-conjugated secondary antibody, as recommended by the manufacturer (Promega Biotec). Cloning: the rat brain cDNA expression library constructed in λZAP from size-selected (that is, >3 kilobase pairs) cDNAs was a gift from Dr K. Angelides. Roughly 3×10^5 recombinants were screened essentially as for λgt11[50], but using *E. coli* strain BB4. Serum (see text) was used at a dilution of 1:500 or 1:1,000 at room temperature overnight. Fourteen antibody-positive clones (λZAP-dynamin-1–14) were plaque-purified to homogeneity. Both autoimmune and immune rabbit antibodies, which were affinity-purified using the fusion protein produced by λZAP-dynamin-1[50], reacted with D100.

Molecular cloning of D100

Two antisera were used in this study. The first was derived from an autoimmune serum identified while screening rabbits before immunization. Serum from one of seven animals stained D100 along with several other bands on immunoblots of calf and rat brain cytosolic extract. Antibody was used after

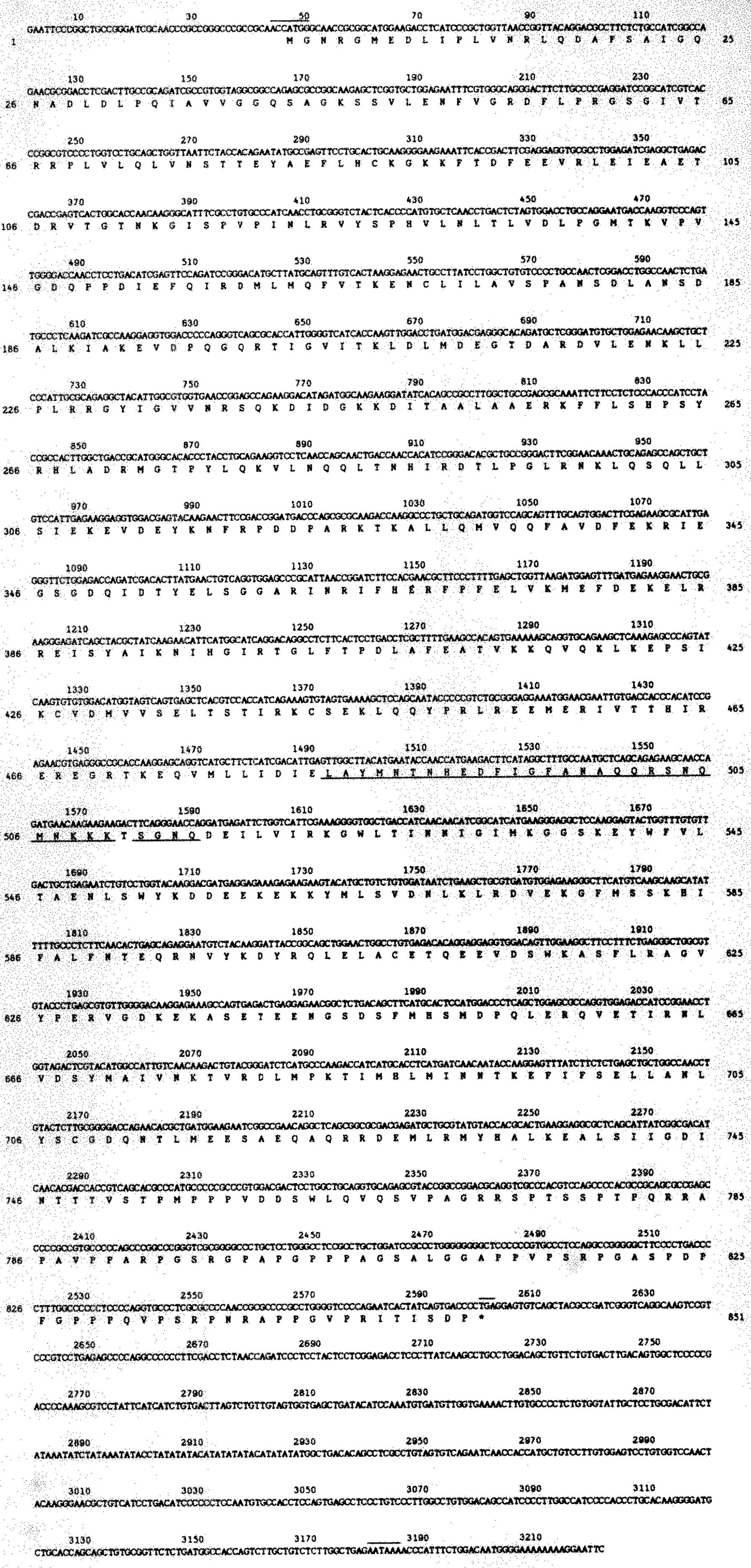

FIG. 2 Nucleic acid and deduced amino-acid sequence of the cDNA insert of clone λZAP-dynamin-1. The putative start (ACCATGG)[16], stop (TGA), and polyadenylation (AATAAA) sequences are overlined. Amino-acid residues confirmed by direct sequencing of bovine D100 are underlined. METHODS. DNA sequencing: The recombinant pBluescript phagemid was excised from clone λZAP-dynamin-1 by the *in vivo* protocol of Short *et al.*[51] and each strand sequenced with Sequenase (US Biochemicals) from a set of overlapping exonuclease III, *Hin*cII, *Pst*I, and *Pvu*II fragments; synthetic oligonucleotide primers were used to fill in gaps. Peptide sequencing: About 500 picomoles of the $M_r \sim 100$K polypeptide of calf brain dynamin (prepared as in ref. 5) was further purified by preparative electrophoresis through a 7% sodium dodecyl sulphate polyacrylamide gel[48], then digested *in situ* in a second, 14% gel with *Staphylococcus aureus* V8 protease with an enzyme:substrate ratio of ~1:400[52]. This gel was electroblotted onto Immobilon-PVDF, which was then stained with Coomassie brilliant blue. A major peptide of $M_r \sim 30$K was excised from the blot and sequenced on an Applied Biosystems Model 470A sequenator, to give the sequence: LAYMNTNHEDFIGFANAQQRSNQMNKKKASGNQ with an initial yield of 106 picomols. This bovine sequence differs from the derived rat sequence after Glu 482 (a likely V8 cleavage site) only by the replacement of an alanine for threonine 511.

FIG. 3 *a*, Alignment of the nucleotide-binding motif of D100 with those from GTP-binding proteins of several families. Dynamin-1, sequence derived from λZAP-dynamin-1 (see Fig. 2); Ras, human Ha-Ras p21 (taken from ref. 42); EF-Tu, *E. coli* elongation factor Tu[53]; SRP54, the M_r 54K subunit of the signal recognition complex[20,21]; Gs alpha, the α subunit of the bovine brain Gs complex[54]. *b*, Alignment of elements I and II of the D100 sequence with those of kinesin heavy chain[9] and three kinesin heavy chain-like proteins[11-14]. The kinesin heavy-chain family is found to contain GTP-binding element II, which is aligned with the comparable region of D100.

a

Dynamin-1	I A V V G G Q S A G K S S V L E	(34-49)	L T L V D L P G M T K V	(132-143)	T K L D	(205-208)
ras	L V V V G A G G V G K S A L T I	(6-21)	L D I L D L A G Q E E Y	(53-64)	N K C D	(116-119)
Gs alpha	L L L L G A G E S G K S T I V K	(43-58)	F R M F D V G G Q R D E	(219-230)	N K K D	(292-295)
SRP54	I M F V G L Q G S G K T T T C S	(104-119)	I I I V D T S G R H K Q	(186-197)	T K L D	(248-251)
EF-Tu	V G T I G H V D H G K T T L T A	(14-29)	A H V D D C P G H A D Y	(76-87)	N K C D	(135-138

b

Dynamin-1	I A V V G G Q S A G K S S V L E	(34-49)	L T L V D L P G M T K V	(132-143)
Kinesin HC	I F A Y G Q T S S G K T H T M E	(88-103)	L Y L V D L A G S E K V	(234-245)
ncd	I F A Y G Q T G S G K T Y T M G	(430-445)	I N L V D L A G S E S P	(576-587)
KAR3	I F A Y G Q T G S G K T F T M L	(470-485)	L N L V D L A G S E R I	(622-633)
BimC	I F A Y G Q T G T G K T Y T M S	(163-178)	L N L V D L A G S E N I	(318-329)

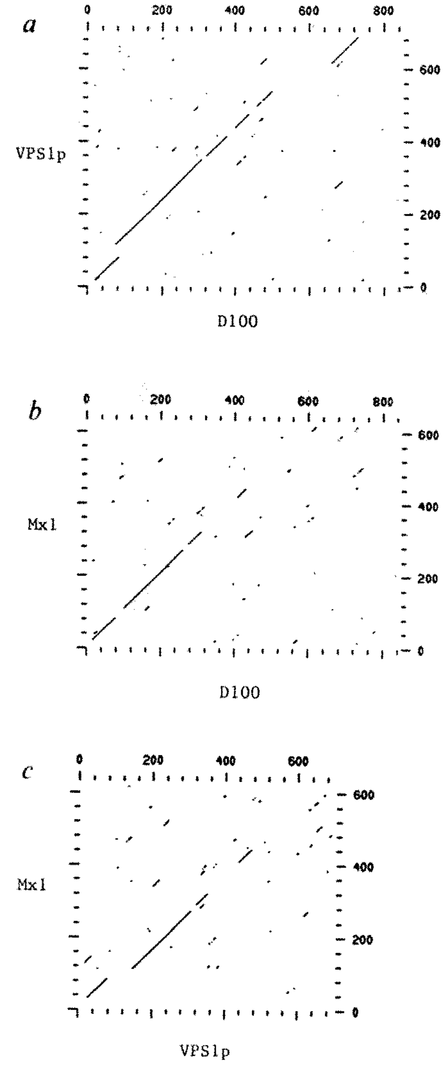

FIG. 4 Dot-plot comparisons of the predicted sequences of the rat D100 polypeptide, yeast VPS1p[33] and murine Mx1 (taken from ref. 27). *a*, D100 versus VPS1p; *b*, D100 versus Mx1; *c*, VPS1p versus Mx1.

METHODS. The following programs of the University of Wisconsin (Madison), Genetics Computer Group Sequence Analysis Software Package (version 6.1) were carried out on a VAX 11/750 computer[26]: FASTA for database searches using the entire λZAP-dynamin-1 cDNA sequence or its derived polypeptide sequence; GAP for alignments; and DOTPLOT for dot-matrix comparison of pairs of sequences. Each of the DOTPLOT profiles was obtained with a window size of 32 and a stringency of 16.

affinity-purification from either electrophoretically purified D100 or bacterially expressed fusion protein (see below). A second rabbit polyclonal antibody was raised (C.A.C. and M. Stachniak, manuscript in preparation). Using this antibody, clones were selected from a rat brain cDNA expression library constructed in λZAP (see legend to Fig. 1). One of these clones was analysed in detail. It was judged to encode D100 by several criteria: (1) the β-galactosidase fusion protein produced by the clone was recognized by affinity-purified anti-D100 antibody preparations from both rabbits (see Fig. 1); (2) the clone contains an open reading frame beginning with a consensus eukaryotic translation initiation sequence (ACCATGG; ref. 16) that predicts an 851-amino-acid polypeptide of M_r 95,928, consistent with the size of the rat brain polypeptide observed by denaturing polyacrylamide gel electrophoresis[5] (Fig. 2); and (3) the cDNA encodes an amino-acid sequence (residues 498-530) nearly identical to that obtained by amino-terminal microsequence analysis of a V8 proteolytic fragment of the bovine brain protein (see legend to Fig. 2). This cDNA clone, which contains a 3,215-base pair insert, was named λZAP-Dynamin-1.

Relationship to other proteins

In view of the nucleotide-dependent interaction of D100 with microtubules, we inspected the predicted amino-acid sequence for features found in other nucleotide- or microtubule-binding proteins. Previous work indicated that dynamin is an ATPase[5]. The consensus sequence element indicative of ATP binding (the 'A-box' or 'element I')[17,18] is GXXXXGKS(T) (single-letter amino-acid code, where X is any amino acid). This was found in the D100 sequence beginning at Gly 38. Surprisingly, two additional elements, DXXG (element II; beginning at Asp 136) and N(T)KXD (element III; beginning at Thr 205), which together with the first element are indicative of a GTP-binding motif[19-21], are also present (see Fig. 3*a*).

Inspection of the D100 sequence revealed no overt similarity to the proposed microtubule-binding motifs of tau[22], MAP 2 (ref. 23), MAP 1B heavy chain[24], or the trypanosome MAP p320 (ref. 25), all of which contain tandem repeats of lengths from 15 to 38 amino-acid residues. No repeated sequence elements were found in the nucleic acid or protein sequences reported here.

We found no extended similarity with kinesin heavy chain or any of the other members of the kinesin superfamily[9-14]. However, there is a sequence of 12 amino acids in D100, containing GTP-binding element II, that is quite similar to a region of the kinesin heavy-chain sequence near its proposed microtubule-binding site[9,10] (Fig. 3*b*).

We searched the Genbank, EMBL, Swissprot and NBRF databases for D100-like sequences using the program FASTA[26]. Two sequences with substantial similarity to D100 were found: the predicted products of the murine *Mx1* and *Mx2* genes[27,28].

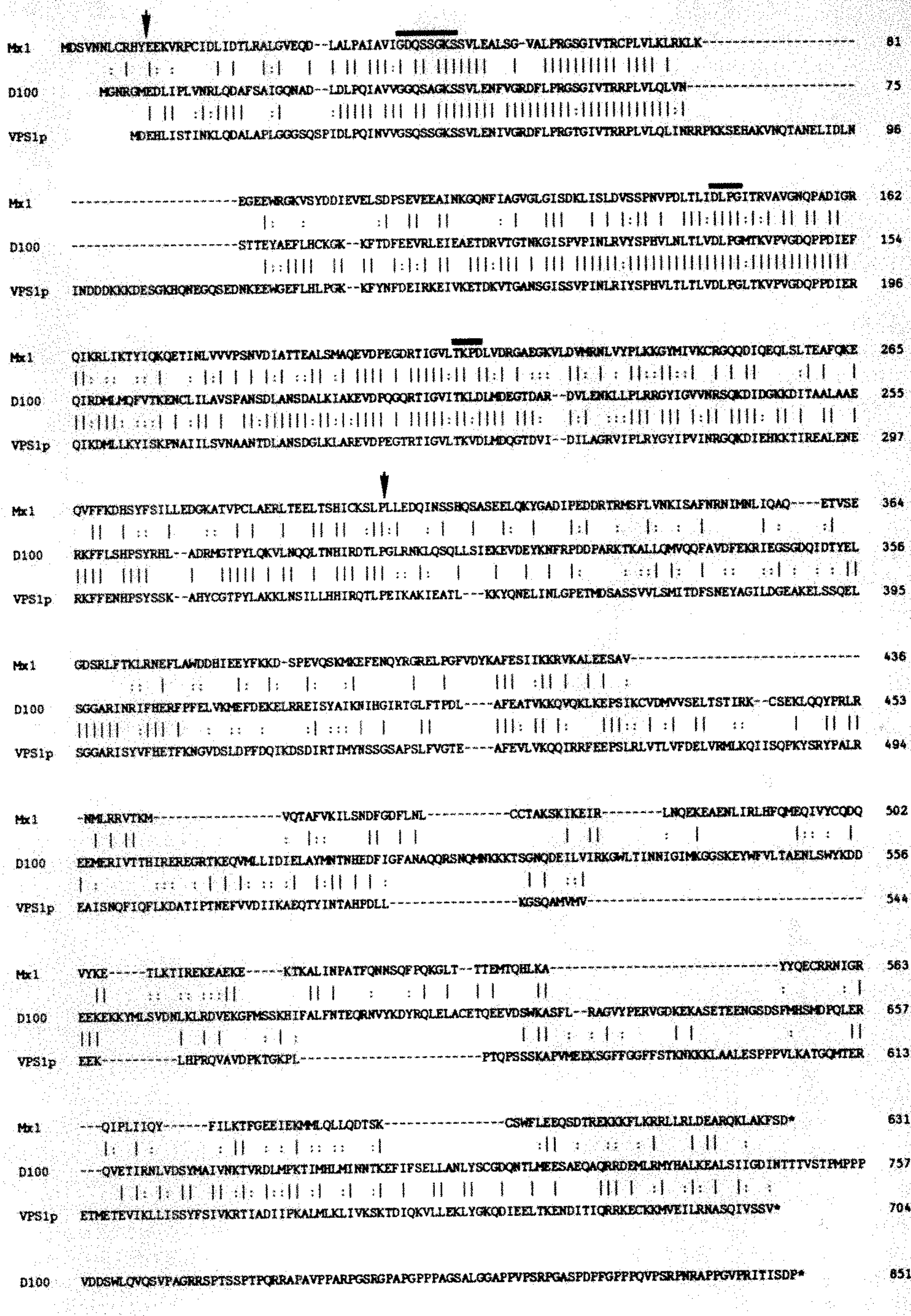

FIG. 5 Alignment of the predicted D100, VPS1p and Mx1 sequences. The sequences were aligned as described in the legend to Fig. 4. Arrowheads mark boundaries of the region of greatest similarity (see text). This region was selected visually, with the aid of the dot-matrix plots shown in Fig. 4. Vertical bars, identity; colons, conservative substitutions. The guanine-nucleotide-binding consensus elements are overlined.

These genes are members of a family encoding a set of interferon-inducible polypeptides of M_r 72–80K, found in mouse[27,28], human[29,30], rat[31] and fish[32]. The best studied of these, and the one that we analysed in detail, is the mouse *Mx1* gene product, which specifically confers influenza virus resistance on transfected mammalian cells in culture[27]. The mechanism of action of the Mx polypeptides is unknown, but there is a GTP-binding motif in the predicted sequences of two human Mx polypeptides[30].

Analysis of the yeast vacuolar protein sorting gene *VSP1* has also revealed clear homology with the *Mx* family[33]. We therefore compared the sequence of D100 with that of the polypeptide encoded by *VSP1*. Extensive similarity was found. We aligned these sequences and carried out dot-matrix comparisons to identify regions of conserved sequence, which appear as diagonal lines (Fig. 4). Overall sequence identity of 32% was seen between D100 and Mx1 and 45% between D100 and VPS1p, as compared with 32% between Mx1 and VPS1p. The most conserved region in all three pairwise comparisons contains the three GTP-binding elements (the consensus positions of which are identical in all three proteins, Fig. 5) and aligns to a 288-amino-acid region between Glu 7 and Pro 294 of the D100 sequence (Fig. 5). D100 and Mx1 show identity at 43% of all aligned positions in this region, D100 and VPS1p 66%, and Mx1 and VPS1p 44%.

Because the Mx proteins of mammalian cells are only expressed following interferon induction[34], we would not have detected these proteins in brain tissue using our anti-D100 antibodies (Fig. 1). But we were interested in the possibility that the initial immune response of our autoimmune rabbit involved Mx. To investigate this question, we expressed mouse Mx1 in *Escherichia coli* as a glutathione-*S*-transferase fusion protein. Antibody, purified using authentic bovine brain D100, bound equally well to the β-galactosidase–D100 and glutathione-*S*-transferase–Mx1 fusion proteins (Fig. 6), consistent with the idea that the antibody represented a response to a previous viral infection. Mx1 is a minor histocompatibility antigen[35], but, to our knowledge, no other autoimmune reactivity with Mx proteins has been reported. We also note that stronger reactivity was observed with the D100 polypeptide than with the fusion proteins (not shown), suggesting that some of the determinants recognized by the antibody were modified in the brain polypeptide.

Functional considerations

The sequence analysis reported here indicates a nucleotide-binding site within D100, as expected from its mechanochemical properties[5]. This site is contained within a domain of $\sim$300 amino acids also found in two other classes of polypeptides, VPS1p and Mx. The predicted sequence of the D100 polypeptide is unrelated to those of the rapidly growing family of kinesin-related proteins, with the exception of the small region we identify surrounding GTP-binding consensus element II (Fig. 3). No other similarity to the $\sim$350-amino acid, force-producing head domain of kinesin, the common structural element in the kinesin family, was observed[9-14]. Although there is no direct evidence that the slightly smaller conserved domain in D100, Mx1 and VPS1p corresponds to a functionally analogous head, this is an appealing possibility.

A surprising result of the sequence analysis was the identification of GTP-binding consensus elements. Earlier work had revealed a microtubule-activated ATPase activity associated with dynamin[5]. GTPase activity was not evaluated, but there have been clear indications that GTP could also be used as a substrate. D100 could be extracted from microtubules with GTP as well as ATP, and GTP extraction is routinely used in D100 purification[5]. We have now found that purified D100 has a potent, microtubule-activated, GTPase activity (unpublished data). This result, along with other information, suggests that GTP is the likely physiological substrate for dynamin. In this context, we note that kinesin can also use GTP, although its affinity for ATP is higher, and therefore ATP seems to be its physiological substrate[2,36]. Kinesin contains GTP-binding consensus element II (Fig. 3), though we find no evidence for element III. This indicates that use of GTP is possible without the third consensus element, which is known from crystallographic studies to be important to base specificity[37,38]. In view of the fact that D100 and kinesin show limited similarity adjacent to element II, an additional role for this region, such as microtubule binding, is also worth considering.

The relationship between D100 and other GTP-binding proteins is, in general, limited to the composition and spacing of the three GTP-binding consensus elements (Fig. 3). However, homology of D100 with Mx1 and VPS1p extends over more than the first 700 amino acids of D100 (Fig. 5). This identifies D100, the Mx proteins and VPS1p as members of a new family of GTP-binding proteins. This family is distinct from the protein synthesis initiation and elongation factors[39], the tubulins[40], the signal-transducing G proteins[41], and the $p21^{ras}$ proteins[42]. Two other families have been proposed, one including components of the signal recognition particle[20,21,43] and the other including proteins involved in secretion[44]. The members of the new family described here are, in general, larger than the members of the other families, and contain a threonine at the first position of the GTP-binding consensus element III (as do the members of the signal recognition particle family), a position shown to be functionally important in $p21^{ras}$ by mutational analysis[42].

The members of the new family are extensively related in their amino-terminal domains, but differ in their lengths and carboxy-terminal domains. This relationship is similar to that between the members of the kinesin and myosin families[45,46], for which it has been proposed that the conserved domains confer common force-producing properties, whereas the variable domains confer functional specificity by directing binding to different intracellular targets. Thus, a simple model predicts that D100, the Mx proteins, and VPS1p are all involved in microtubule-associated motility. How this pertains to the function of the Mx proteins is unclear. In the case of VPS1p, an involvement of microtubules can be imagined, as anti-microtubule drugs result in vacuolar fragmentation[47]. We note that, despite the overall conservation of the amino-terminal domain, VPS1p contains a sequence between GTP-binding elements I and II which is absent from D100 and Mx1 (Fig. 5). According to our model, this segment could conceivably alter the interaction of VPS1p with microtubules.

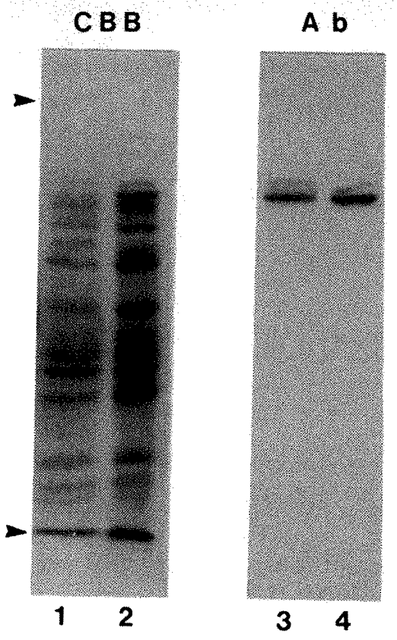

FIG. 6 Cross-reactivity of autoimmune antibody with D100 and Mx1 fusion proteins. Lanes 1, 3, Isopropyl thiogalactoside-induced plaques from clone λZAP-dynamin-1; lanes 2, 4, liquid lysate from isopropylthiogalactoside-induced plasmid pGEX-Mx1. The top of the blot and the dye front are marked by arrowheads. Proteins were visualized as described in the legend to Fig. 1.

METHODS. Electroblotting and autoimmune antibody purification steps were as described in Fig. 1. Construction of pGEX-Mx1: the *Afl*II site of the 3,700 bp *Afl*II–*Eco*RI fragment of pMx34 (ref. 27) was converted to a *Bam*HI site by ligation to a double-stranded adaptor: 5′-GATCCGGCAACGAAGGTACCATGGGAATTC-3′ and 5′-TTAAGAATTCCCATGGTACCTTCGTTGCCG-3′, and the fragment was subcloned into a vector cut with *Bam*HI and *Eco*RI (pBluescript SK+), then removed as an *Nco*I–*Eco*RI fragment, using the *Nco*I site from the adaptor. This fragment was ligated to a modified plasmid, in which an *Nco*I site had been inserted between the *Bam*HI and *Eco*RI sites of pGEX-2T (ref. 55) with a double-stranded adaptor (Boehringer-Mannheim). This final construct, which contains the entire *Mx*1 coding sequence in-frame with a glutathione-*S*-transferase gene, was named pGEX-Mx1.

In addition to the most highly conserved amino-terminal region of $\sim$300 amino acids, sufficient similarity exists between D100, Mx1 and VPS1p that they can be readily aligned up to the carboxyl termini of Mx1 and VPS1p. Beyond this point, D100 contains a distinct carboxy-terminal extension of $\sim$100 amino acids. This region is very proline-rich (32%) and polar (isoelectric point, $pI = 12.5$), and is thus likely to be on the surface of the molecule in a relatively extended conformation. In view of its basicity, it may also be involved in microtubule binding. Two microtubule-binding sites are predicted for D100 from the ability of dynamin to crosslink microtubules[5]. Assuming that one microtubule-binding site lies within the conserved amino-terminal domain of D100, the other site is likely to lie at the other end of the polypeptide and could be represented by the carboxy-terminal extension. Its absence from Mx1 and

VPS1p suggests that, although these proteins are mechanistically related to dynamin, there are likely to be important functional differences as well.

Thus, analysis of the primary structure of D100 supports its biochemical identification as a component of a mechanochemical protein, and has revealed substantial homology with a new family of proteins. The variety of contexts in which these proteins have been identified suggests that this family mediates a wide range of novel microtubule-based cellular functions.

Note added in proof: The sequence data reported will appear in the EMBL, GenBank, and DDBJ Nucleotide Sequence Databases under the accession number X54531. □

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LETTERS TO NATURE

Eötvös experiments, lunar ranging and the strong equivalence principle

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THE strong form of Einstein's equivalence principle, which dictates that gravitational binding energy will suffer the same acceleration in a uniform gravitational field as all other forms of matter and energy, can be tested by comparing the accelerations of the Earth and the Moon towards the Sun. If the Earth's gravitational binding energy, which contributes ~5 parts in 10^{10} of its total mass, does not gravitate in the same way as other kinds of mass and energy, the Moon's orbit will be distorted in a characteristic way with an amplitude large enough to be detected by lunar laser-ranging data. But as Nordtvedt[1] has pointed out, such a test is accurate only to the extent that no interfering force, such as a very feeble composition-dependent 'fifth force', fortuitously cancels a gravitational binding energy anomaly. Here we review laboratory upper limits on 'fifth forces' to set an upper limit of 1 part in 10^{12} (95% confidence level) on the Earth–Moon acceleration difference due to non-gravitational forces. Existing lunar-ranging data then verify the strong equivalence principle to an accuracy of 3%, and improved analyses of the lunar data should bring this precision down to the 0.2% level.

Einstein's equivalence principle, one of the experimental cornerstones of the general theory of relativity, has been tested with great precision in laboratory experiments of the torsion-balance type pioneered by Eötvös. But these experiments test only the 'weak' version of the principle, because the gravitational binding energy of laboratory objects, at no more than 10^{-27} of their mass, is immeasurably small. But tests of the strong equivalence principle are now possible because the relative motion of Earth and Moon can be determined with great accuracy by measuring the round-trip travel time of light pulses emitted by Earth-based lasers and reflected back to Earth by corner cubes placed on the Moon by the Apollo astronauts. Such lunar-ranging measurements have been carried out during the past two decades at several observatories, but require careful analysis to correct for a number of possible systematic errors. Analyses[2,3] of the lunar-ranging data obtained up to 1976 established that the accelerations of Moon and Earth toward the Sun are identical to within 14 parts in 10^{12} (95% confidence level), in agreement with the strong equivalence principle and with the predictions of general relativity. It is expected[1] that refined analyses including more recent data will test this equality to a precision of ~1 part in 10^{12}.

To what accuracy can we be sure that some other interaction, a fifth force, does not affect the differential acceleration of the Earth and Moon, and mask a violation of the strong equivalence principle? It is necessary to consider both forces mediated by vector (spin = 1 $\hbar$) and scalar (spin = 0) particles. Either will generate a potential between two point objects a distance r apart of the form

$$V_{12}(r) = \pm\frac{g_5^2}{4\pi}(q_5)_1(q_5)_2\frac{e^{-r/\lambda}}{r} \tag{1}$$

where g_5 is the coupling strength (the + and − signs refer to vector and scalar interactions respectively), λ is the interaction range, and q_5 is the test body 'charge', determined by the particular form of the interaction. Such an interaction would cause the Earth and Moon to fall toward the Sun with accelerations a that differ by

$$\frac{\Delta a}{g_{\mathrm{Sun}}} = \mp \frac{g_5^2}{4\pi G}(q_5/m)_\odot \Delta(q_5/m)\, e^{-R/\lambda}\left(1+\frac{R}{\lambda}\right) \qquad (2)$$

where g_{Sun} is the mean gravitational acceleration of the Earth toward the Sun, G is the newtonian gravitational constant, R is the mean radius of the Earth's orbit around the Sun, $(q_5/m)_\odot$ is the 'charge'-to-mass ratio of the Sun, and $\Delta(q_5/m)$ is the difference in 'charge'-to-mass ratios of the Earth and Moon. (For simplicity, we assume that λ is large compared to the radius of the Sun.)

Any such interaction affecting the Earth-Moon differential acceleration would also affect the differential acceleration of laboratory-sized objects, and thus could be detected by torsion-balance experiments used to test the weak equivalence principle. In essence, these experiments compare the accelerations of two different test bodies placed in a gravitational field. If all extraneous forces on the test bodies are eliminated and if the equivalence principle holds, then these accelerations will be identical, regardless of the material of which the test body is constructed. On the other hand, a 'fifth force' will produce an effect that looks like a violation of the equivalence principle. The three most precise such tests are the experiments by Dicke *et al.*[4] and Braginksy and Panov[5] (which compared the acceleration of Al and Au, and Al and Pt toward the Sun, respectively) and by the Eöt-Wash group[6] (which compared the acceleration of Be and Cu and of Be and Al toward the Earth). All of these experiments obtained a null result, consistent with the weak equivalence principle, and each sets upper bounds on possible interactions affecting the Earth-Moon-Sun system.

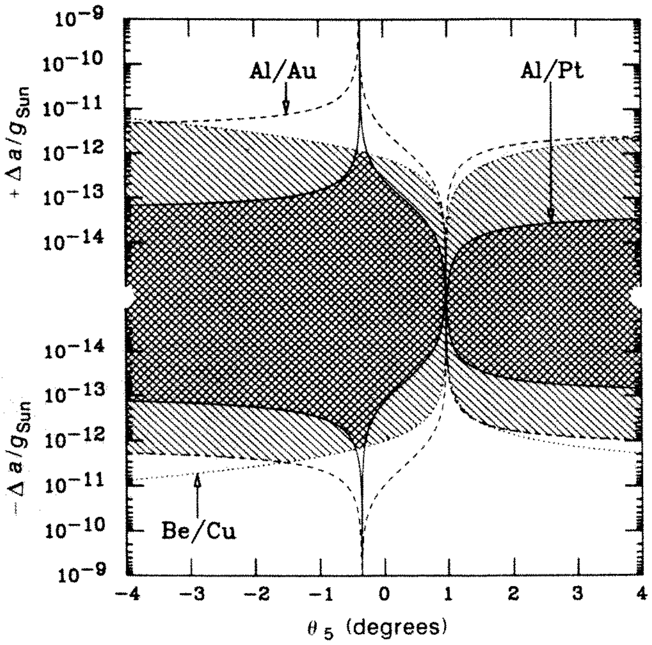

FIG. 1 Maximum allowed contribution (at 2σ) of an infinite-ranged vector force to the Earth-Moon differential acceleration toward the Sun. The lightly shaded region is consistent with the Princeton and Eöt-Wash results; the heavily shaded area with the Moscow and Eöt-Wash data. The zero occurs because the Earth-Moon charge difference vanishes at $\theta_5 \approx 1°$. The poles occur at values of θ_5 where the charge difference vanishes for the Al/Au and Al/Pt test body pairs used in the Princeton and Moscow experiments. Outside the range of this plot, the constraints from the solar experiments are nearly independent of θ_5; the Eöt-Wash terrestrial result has poles where the test-body charge difference vanishes (at $\theta_5 \approx -11°$ for Be/Cu) and where the Earth's charge vanishes (at $\theta_5 \approx -64°$), and a zero (at $\theta_5 \approx -49°$) where the charge of the Sun vanishes. The joint constraints for $|\theta_5| > 4°$ are at least as stringent as those shown in this plot.

To extract these bounds one must consider the possible charges that appear in equations (1) and (2). The most general charge for a vector interaction of stable electrically neutral matter is

$$q_5 = B\cos\theta_5 + L\sin\theta_5 \qquad (3)$$

where B and L are the numbers of baryons and leptons, respectively, and θ_5 is an arbitrary parameter that specifies the precise form of the charge. It is more difficult to specify the scalar charge of a material. Although the scalar couplings may be simple in terms of the fundamental fields (for example, the expectation value of the total number of quarks and anti-quarks), the total scalar charge of a neutral atom reflects its complex structure and will depend in a complicated fashion on the atomic and nuclear binding energies. Following the ideas of Peccei, Sola and Wetterich[7], we characterize the scalar charge with the phenomenological expression

$$q_5 \approx \mu + \varepsilon_B|B| + \varepsilon_L|L| \qquad (4)$$

where μ is the mass in atomic mass units (a.m.u.) and $|\varepsilon_B|$ and $|\varepsilon_L|$ are both much less than one.

For our purposes, the scalar and vector charges are very similar. In both cases a fifth force will produce an acceleration difference that is linear in $\Delta B/\mu$ and $\Delta L/\mu$ (the difference in baryons/a.m.u. or leptons/a.m.u. of the Earth and Moon, or of the two test bodies in a torsion balance). Because any given pair of test bodies may accidentally have a vanishing charge difference, at least two laboratory Eötvös experiments with quite different test bodies are required to rule out the possibility that a non-gravitational force affects the Earth-Moon differential acceleration toward the Sun. The Princeton and Moscow experiments have very similar test bodies and, in this respect, are not really independent. By combining their Al/Au and Al/Pt results with Eöt-Wash Be/Cu and Be/Al data, however, we can place firm upper limits on any Earth-Moon differential acceleration owing to composition-dependent forces. We discuss the case of a vector interaction (which must couple to a linear combination of B and L) in detail. Using accepted values for the elemental compositions of the Sun[8], Earth[9,10] and Moon[11], we deduce

$$(B/\mu)_{\mathrm{Earth}} = 1.000759(10) \qquad (L/\mu)_{\mathrm{Earth}} = 0.4869(10) \qquad (5)$$

$$(B/\mu)_{\mathrm{Moon}} = 1.000603(15) \qquad (L/\mu)_{\mathrm{Moon}} = 0.4942(10) \qquad (6)$$

$$(B/\mu)_{\mathrm{Sun}} = 0.9942 \qquad (L/\mu)_{\mathrm{Sun}} = 0.8592 \qquad (7)$$

and calculate, as a function of θ_5, the maximum differential acceleration of the Earth and Moon allowed by the Princeton, Moscow and Eöt-Wash results. (The errors in equation (5) and equation (6) come from assuming that the Fe contents of the Earth and Moon are known to 10% and 30%, respectively. It is the difference in the Fe content that largely accounts for $\Delta B/\mu$ and $\Delta L/\mu$ for the Earth-Moon system. The only effect of these uncertainties is to shift the zero in Fig. 1 by a fraction of a degree, and to increase the uncertainty in Δa by ~20%, which is included in the limits shown in Fig. 1. No errors are quoted for the Sun because these (small) errors have a negligible effect on our conclusions. The results for a vector interaction are shown in Fig. 1. At two standard deviations, the largest $\Delta a/g_{\mathrm{Sun}} = \Delta m/m$ consistent with our results and those of the Princeton or Moscow groups is $\sim 5\times10^{-12}$ or $\sim 1\times10^{-12}$, respectively. The corresponding constraints for a scalar force are essentially identical to those discussed here for a vector interaction. This limit on the differences between fifth-force accelerations is equal to the expected precision with which the Earth-Moon differential acceleration can ultimately be extracted from existing lunar-ranging data, and is a factor of ~14 less than the uncertainties in existing analyses of this quantity.

As gravitational binding energy makes a 5.1×10^{-10} contribution to the Earth's mass (our result differs slightly from the value 4.6×10^{-10} quoted in refs 1 and 2 because we use a realistic model for the Earth's density rather than assuming it to be constant), our upper limit on non-gravitational-acceleration differences coupled with the 1976 lunar-ranging analysis unambiguously establishes that gravitational binding energy has the same acceleration in a gravitational field as other forms of matter and energy to a precision of 3% (95% confidence level), and will allow the anticipated improvements in the lunar-ranging analysis to test the strong equivalence principle to a precision of 0.2%. The Eöt-Wash results[6] can probably be improved by a factor of ~10 by better seismic and thermal isolation of the torsion balance. At this point one could, by combining the Eöt-Wash results with the existing Moscow data, detect non-gravitational Earth-Moon acceleration differences with a sensitivity of $\sim 10^{-13} g_{\mathrm{Sun}}$, which lies below the anticipated precision of the lunar-ranging data. This may provide further motivation for future space experiments to test post-newtonian gravitational effects. ☐

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Synthesis of the defect perovskite series $LaCuO_{3-\delta}$ with copper valence varying from 2+ to 3+

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MANY important questions about the origin of superconductivity in the high-temperature copper oxide superconductors could be addressed by the study of a copper oxide compound exhibiting a wide range of oxygen stoichiometry while maintaining the basic perovskite structure. A number of compounds are known in which copper occurs formally as Cu^{3+} (refs 1, 2), but most are structurally unrelated to the superconductors, so that a direct comparison of their chemical and physical properties is not appropriate. Here we report the synthesis, structure and preliminary characterization of a new oxygen-deficient perovskite series, $LaCuO_{3-\delta}$. Although superconductivity has not been observed in this system, its oxygen stoichiometry range, $0.0 < \delta < 0.5$, is the widest yet found in a copper oxide compound, allowing the formal copper valence to be varied from 2+ to 3+. This series may thus serve as a model system with which to address such questions as the nature of the Cu/O hole states in the superconductors, and the way in which magnetic and transport properties vary with carrier concentration.

$LaCuO_3$ was first prepared by Demazeau *et al.*[3] at 900 °C and 65 kbar using $KClO_3$ as an oxygen buffer. Its X-ray powder diffraction pattern was successfully indexed as a rhombohedrally distorted perovskite cell and it was reported to be fully stoichiometric and to display metallic properties down to 77 K. Because of the very high pressure and other complexities of its synthesis, however, there has been no further characterization of this important phase. In searching for milder synthetic conditions, we discovered a new series of oxygen-defect $LaCuO_{3-\delta}$ perovskites, with $0 \leq \delta \leq 0.5$.

Phases in the $LaCuO_{3-\delta}$ system were prepared at 900 °C at oxygen pressures of 0.2–1 kbar using coprecipitated La and Cu hydroxide precursors. The precursors were decomposed by slow heating to 650 °C under oxygen before reacting them in high-pressure oxygen. Exclusion of CO_2 and other organic impurities from the starting materials and an oxidizing atmosphere was necessary to obtain single-phase, homogeneously oxidized products. Compositions were determined by thermogravimetric analyses with an accuracy in δ of ±0.02. High-resolution X-ray powder diffraction data for the various phases in the $LaCuO_{3-\delta}$ system were collected at the Brookhaven National Synchrotron Light Source. Structural refinements were performed with a modified Rietveld program (S. La P., J. B., R. B., D. C. and B. S., manuscript in preparation).

The phase and crystallographic data for $LaCuO_{3-\delta}$ are summarized in Fig. 1. These phase fields were obtained from samples prepared directly, or modified by reduction of a $\delta = 0.1$ composition in N_2 or O_2 (1 atm) at 300–750 °C. Thus Fig. 1 is not a true phase diagram, because the data were not obtained under equilibrium conditions. We have found that it is possible to change the ranges somewhat by varying the preparation or processing conditions. The evolution of structure as δ is varied is given in Fig. 2. We observe three distinct ordered arrangements of oxygen (or vacancies). A tetragonal perovskite with a homogeneity range $0 \leq \delta \leq 0.2$ occurs at 900 °C and $P_{O_2} = 0.4$–1 kbar. Samples for which $\delta = 0$ were obtained for $P_{O_2} \geq 0.5$ kbar, but in general, oxygen stoichiometries were dependent on several factors, including the homogeneity and purity of the starting materials. At lower oxygen pressures, $P_{O_2} = 0.2$–0.4 kbar, the degree of oxidation decreases, leading to the formation of a monoclinic phase stable over the interval $0.2 \leq \delta \leq 0.4$. Both forms are metallic, with room-temperature resistivities of 100 μΩ cm and

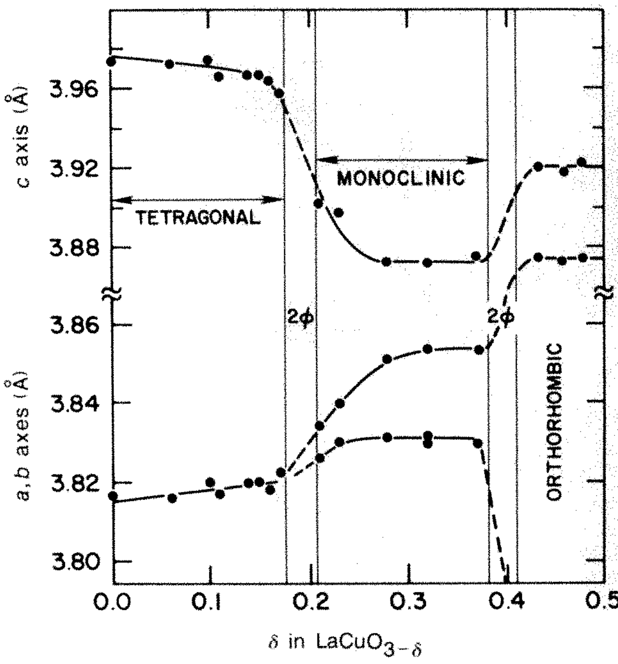

FIG. 1 The variation of the perovskite subcell parameters with oxygen content in $LaCuO_{3-\delta}$. The subcell *a* axis of the orthorhombic phase is 3.70 Å. 2*ϕ*, approximate two-phase region discussed in the text.

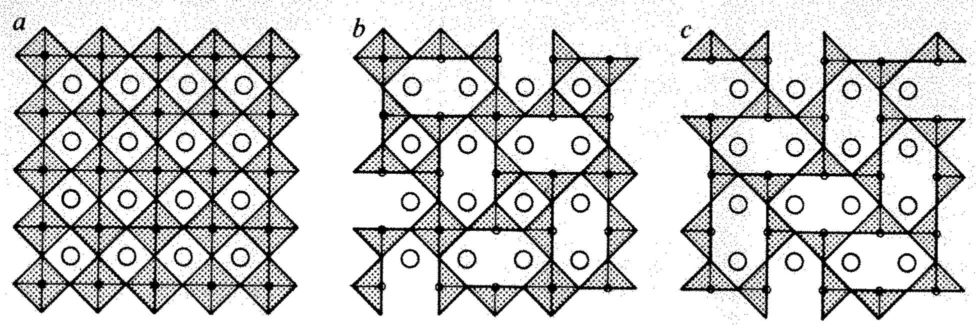

FIG. 2 The evolution of oxygen-vacancy ordering in the $LaCuO_{3-\delta}$ perovskite system. The structures shown are the (001) plane projections for: *a*, the tetragonal phase with $\delta=0$; *b*, the idealized monoclinic perovskite with $\delta=0.4$; *c*, the idealized orthothombic perovskite with $\delta=0.5$. La, large open circles; Cu-O polyhedra, shaded.

300 μΩ cm for $\delta=0.14$ (tetragonal) and $\delta=0.33$ (monoclinic), respectively. Superconductivity has not been observed in either case down to 4.2 K. Lastly, an orthorhombic perovskite can be prepared by heating the tetragonal form to 700-800 °C in N_2. This phase has a homogeneity range between $0.43 \leq \delta \leq 0.5$ and is an insulator.

The powder X-ray diffraction profiles of the three phases observed in this system are shown in Fig. 3. The tetragonal perovskite with $0.0 \leq \delta \leq 0.2$ exhibits the simplest diffraction pattern with no observable supercell reflections. The diffraction pattern of the tetragonal $\delta=0$ phase is readily distinguishable from the rhombohedral form[3]. All three of the phases contain this basic perovskite pattern, but as oxygen vacancies are created ($\delta>0.2$) supercell reflections begin to appear (Fig. 3*b*). In the monoclinic phase, these are related to the perovskite subcell by approximately $\sqrt{5}a \times a \times \sqrt{5}a$. The diffraction pattern of the third phase (Fig. 3*c*) can be indexed using an orthorhombic cell with lattice parameters related to those of the perovskite subcell by approximately $\sqrt{2}a \times 2\sqrt{2}a \times a$. As is apparent in Fig. 3, the samples are virtually free of secondary phases and in the best cases, no impurity phases could be detected to the limit of our diffractometer (~3%) or in the synchrotron data (~0.5%).

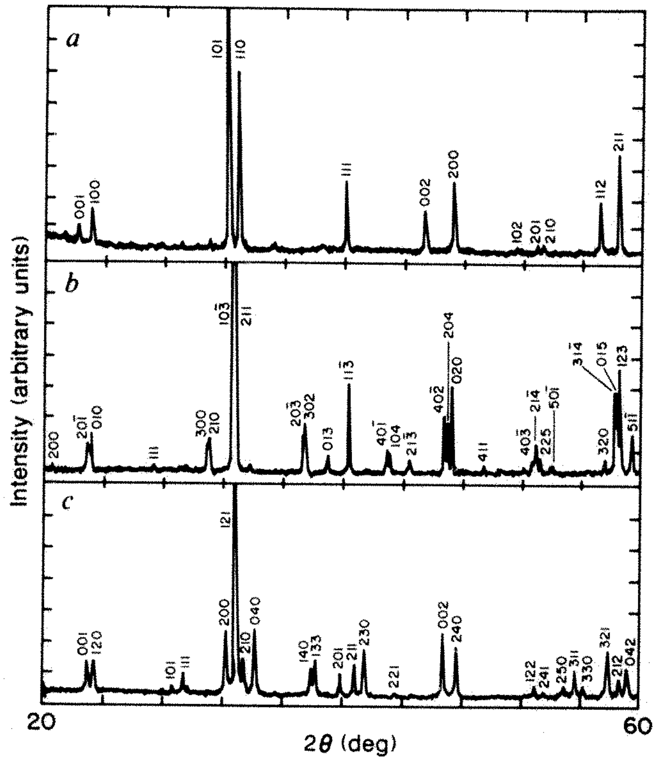

FIG. 3 The powder X-ray diffraction profiles in the region $2(\theta)=20-60°$ of the three phases observed in the $LaCuO_{3-\delta}$ system, with Miller indices indicated. *a*, tetragonal perovskite with $\delta=0$, *b*, monoclinic perovskite with $\delta=0.33$; *c*, orthorhombic perovskite with $\delta=0.5$. For clarity, not all of the Miller indices are shown for the monoclinic phase.

The structure of the $\delta \approx 0$ tetragonal phase (Fig. 2*a*), is a simple tetragonal distortion of a cubic perovskite with $a=3.81875(5)$ Å and $c=3.97270(7)$ Å, space group *P4/m*. This results from a slight Jahn-Teller-like elongation of the CuO_6 octahedra, in which there are four in-plane Cu-O bonds at 1.909 Å, and two apical Cu-O bonds at 1.986 Å. This is in contrast to the rhombohedral form in which all Cu-O distances are equal at 1.94 Å (ref. 3). The unit-cell volume of the tetragonal phase is ~5% larger than that of the rhombohedral form[3], which suggests that the latter is the high-pressure and the former the low-pressure polymorph of $LaCuO_3$. Webb *et al.*[4,5] have reported that a tetragonal phase of $LaCuO_3$ with lattice parameters $a=5.431$ Å and $c=7.84$ Å can be prepared by heating the rhombohedral form to 408 °C. Not all of the lines in the powder pattern of this material could be accounted for, however, and there was in general a very poor fit of the observed and calculated lines. Furthermore, no attempt was made to measure the oxygen content and it seems likely that this material may be non-stoichiometric, because one would expect a Cu^{3+} material to evolve oxygen at this temperature. There are no obvious similarities between the reported diffraction lines and those of the tetragonal phase of $LaCuO_3$ reported here.

Below the phase field of the tetragonal form, oxygen-vacancy ordering leads to a monoclinic perovskite (Fig. 2*b*). The vacancies order along pseudo-cubic {201} axes, giving rise to a new $\sqrt{5}a_p \times a \times \sqrt{5}a_p$ cell with lattice parameters $a=8.62884(5)$ Å, $b=3.83076(2)$ Å and $c=8.65149(5)$ Å for $\delta=0.33$. The synchrotron diffraction peaks of this phase are extremely sharp (~0.025° full width at half maximum) so that the monoclinic distortion from orthorhombic symmetry can be readily distinguished, although it is very small ($\beta=90.214(4)°$). For the expanded cell the unit-cell composition is $La_5Cu_5O_{13.35}$. The structure of this phase closely resembles that of $BaLa_4Cu_5O_{13+x}$ (ref. 6), although the latter is tetragonal. It comprises one-dimensional chains of CuO_6 octahedra oriented along *b* which share corners with four CuO_5 square pyramids in the (010) planes. The CuO_5 pyramids share corners with four other square pyramids but are oriented with their basal faces at 90° to one another. In contrast to $BaLa_4Cu_5O_{13}$, the non-idealized structure (S. La P., J. B., R. B., D. C. and B. S., manuscript in preparation) is significantly distorted from tetragonal symmetry, and the Cu—O—Cu bonds adjoining octahedra and pyramids are bent considerably below the ideal bond angle of 180°.

The third phase observed in the $LaCuO_{3-\delta}$ system appears at $\delta=0.43$. Rietveld refinement shows that oxygen-vacancy ordering in the (001) plane, which is complete at $\delta=0.5$, creates a cell with lattice parameters related to those of the pseudo-cubic perovskite by $a \simeq \sqrt{2}a_p$, $b \simeq 2\sqrt{2}a_p$ and $c \simeq a_p$. Cell parameters obtained from the refinement are $a=5.5491(1)$ Å, $b=10.4782(2)$ Å and $c=3.87956(7)$ Å for a sample of composition $LaCuO_{2.53}$. The idealized structure shown in Fig. 2*c* is isostructural with $CaMnO_{2.5}$ (ref. 7) and is entirely composed of corner-shared CuO_5 pyramids which form pairs by sharing oxygen vacancies in the (001) planes.

As oxygen is removed from the tetragonal $\delta=0$ phase, two CuO_5 pyramids and two sites that are formally Cu^{2+} are created in the form of square-pyramidal pairs for each vacancy inserted.

The result is that Cu is found only in octahedral and/or square-pyramidal coordination throughout the homogeneity range of $LaCuO_{3-\delta}$, and that the number of holes (Cu^{3+} sites) will be formally equal to the number of octahedral coordination sites in the system. This aspect of the structure means that the 3+ and 2+ valence states of Cu in $LaCuO_{3-\delta}$ are 'tagged', a feature which can be used to great advantage in unravelling the electronic structure of the cuprates. For example, we have exploited this to resolve the mystery of the 'missing' electron paramagnetic resonance in the superconducting cuprates[8,9]. As a result of these features and of the wide range of copper valence attainable in this system, this may be an ideal system with which to probe the subtle interplay of crystal-chemical and physical properties, to examine and compare the normal-state properties of the superconducting and related cuprates, and to study the evolution of the electronic properties at higher carrier concentrations. A detailed study of the effects of systematic valence changes on the electrical and magnetic properties in the $LaCuO_{3-\delta}$ system will be reported shortly. □

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Large bubble-like features ordered on a macrolattice in helium-implanted gold

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IMPLANTATION of metals at temperatures near ambient with helium ions can result in the formation of an ordered array—a superlattice—of small (~2 nm in diameter) helium bubbles[1-11]. The superlattice has the same symmetry as the atomic lattice of the metal but with a lattice spacing about 20 times greater. We now report the observation, in helium-implanted gold, of an ordered array—a 'macrolattice'—of artefacts ~60 nm in diameter, which we believe also to be helium bubbles. Again, the macrolattice has the same symmetry as the gold lattice, but the lattice spacing is about 400 times larger. The coexistence in a single specimen of three separate lattices of very similar structure but which differ so markedly in scale provides a new perspective on ordering phenomena. In particular, an understanding of the processes that drive bubble ordering may provide more general insight into the development of microstructures in metals during ion irradiation[12]. Such information is of value for the production of wear- and corrosion-resistant surfaces by ion implantation[13,14] and the development of materials with appropriate erosion and gas-loading properties for use in future nuclear-fusion reactors[15,16].

To produce a bubble superlattice the helium is implanted in the metal at a temperature $<0.3\,T_m$, where T_m is the melting temperature of the metal. For implantation at these low temperatures the bubbles are overpressured[15,17], in the sense that the surface tension of the metal alone is not sufficient to contain the gas pressure. As a consequence, the crystal lattice surrounding each bubble is under considerable stress. In most studies the helium energy is ~30 keV with the ion beam incident along the normal to the target surface. Under these conditions, the helium concentration initially increases rapidly with depth, reaching a maximum at a depth ~100 nm; thereafter the concentration falls rapidly. Thin sections, typically ~60 nm thick, are taken from the implanted surface by thinning the foil to perforation from the rear surface ('back thinning') using chemical electropolishing. In face-centred-cubic metals, the bubble lattice has the same symmetry as the atomic lattice of the host metal and a lattice constant of 6-8 nm. Typical bubble diameters are ~2 nm. A high-magnification transmission electron micrograph of helium bubbles in copper, the most extensively studied of the face-centred-cubic metals, is shown in Fig. 1. It is rarely possible to image the very small superlattice bubbles so clearly in the presence of the high levels of gas, strain and damage in the implanted layer. But even in cases where the bright-field micrographs are ambiguous, the occurrence of satellite reflections around matrix spots in electron diffraction patterns can often provide conclusive evidence of superlattice formation[4].

A previous study showed that a bubble superlattice could be formed in gold[6] and this work is a continuation of that study. To obtain a more uniform helium concentration extending deeper into the target, the helium was implanted at higher energy (160 keV) and at an oblique angle (60° to the surface normal). The helium was broadly distributed about a maximum concentration located at a depth of ~250 nm. Thin sections were prepared by backthinning using argon-ion-beam milling. A gas-bubble superlattice was identified at high magnification in a transmission electron microscope (Philips EM 420 ST). The micrographs and diffraction patterns obtained (not of sufficient quality to be reproduced usefully here) enabled the superlattice parameters to be determined. The average bubble diameter of ~2 nm and lattice constant of ~8 nm are consistent with those found previously[6]. It was at low magnification, however, that the new features were discovered.

The structure in these same gold specimens is dominated by a mesh-like array (Fig. 2), suggestive of a three-dimensional lattice with a periodicity much larger than that of the superlattice. It is made up of rows of artefacts in the two ⟨220⟩ directions that are normal to the direction of the electron beam. We believe that the artefacts defining the mesh are bubbles because: (1) the images have the appearance of large bubbles under a variety of imaging conditions including imaging through the thicker regions of specimens in a 300-keV microscope, (2) individual

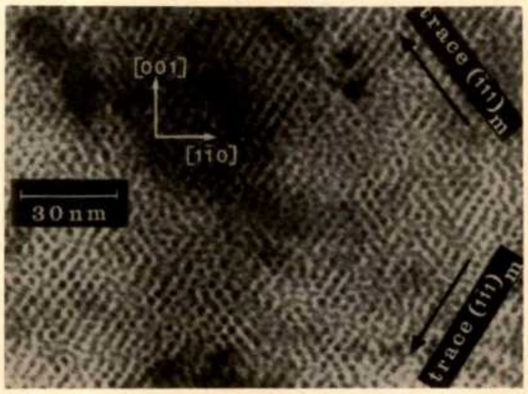

FIG. 1 A transmission electron micrograph of helium gas bubbles in copper following implantation with 4×10^{21} 30-keV $He^+\,m^{-2}$ at 300 K. The electron beam is along [110] in the atomic lattice of the copper and is normal to the plane of the bubble layer and specimen surface. The bubbles are ordered on a superlattice. Principal directions of the copper lattice are indicated. The orientation of bubble rows relative to these directions forms part of the evidence that the bubble superlattice has the same symmetry as the metal lattice.

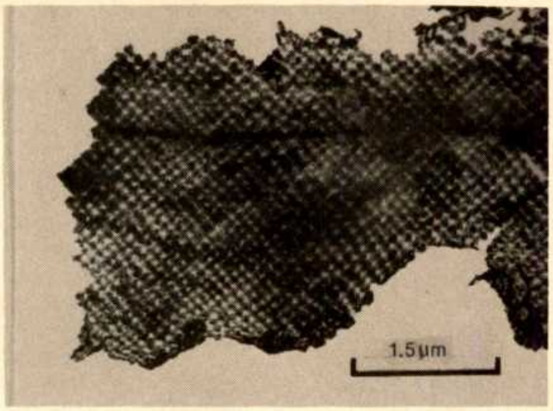

FIG. 2 A transmission electron micrograph of artefacts defining a two-dimensional mesh, in a (100) gold grain following implantation with 1×10^{22} 160-keV He^+ m^{-2} at 280 K. The electron beam is along [100] in the atomic lattice of the gold. Although the artefacts when imaged (as here in the thinnest regions of the specimen) do not have the appearance of bubbles, a variety of observations (see text) have led to the conclusion that they are the remnants of large helium bubbles arranged on a macrolattice.

artefacts in dark-field images have a bubble-like shape defined by thickness extinction contours (approximately circular) of different radii, (3) there are patches in which all the artefacts are larger than those in the surrounding regions (Fig. 3). This is readily explained if the artefacts are bubbles and difficult to explain if they are not. The pressure of the gas in the cavity of an overpressured bubble exerts a large stress on the surrounding metal. Suppose a particular bubble, because of favourable local conditions, were to grow at a rate faster than the average and cause a doming of the specimen surface. A component of tensile stress in the direction of the surface normal would be imposed on the matrix surrounding each of the neighbouring bubbles—a stress which would be expected to enhance bubble growth[18,19]. The correlated growth of bubbles in a patch caused by this mechanism could be described in terms of a lowering of the elastic energy resulting from the group of bubbles cooperating to distort the surface. Cooperative growth of this kind is expected only for large bubbles lying close to the surface. For small bubbles deep in the metal, the interaction between bubbles may act to inhibit bubble growth[9].

Typical bubbles have a diameter of ~70 nm and a spacing of ~105 nm measured along ⟨220⟩ rows. The spacing is a factor of about 20 times greater than that found for the bubble superlattice in gold[6] and is approximately twice the spacing of the coarsest void lattice that has been produced by ion irradiation in a pure (single element) face-centred-cubic metal. (Void lattices with large spacings are produced by irradiation[20] at elevated temperatures $\sim 0.4\,T_m$). This is the first observation of bubble ordering on such a large scale. The result is surprising not only because of the large spacing but also because the macrolattice has a high degree of perfection, extends throughout the specimen and was formed in heavily implanted metal at low temperature $(\sim 0.22\,T_m)$ where vacancies have very limited mobility. The close-packed directions in the bubble macrolattice are parallel to the close-packed directions, ⟨220⟩, of the gold atoms in the (100) surface plane. The specimen thickness is estimated to be $\leqslant$100 nm and this allows just one (100) bubble plane to be contained in the depth of the specimen. For a single plane of bubbles, the directions of the bubble rows are consistent with the macrolattice having a face-centred-cubic symmetry and an alignment parallel with the atomic lattice of the gold. The small bubbles forming the superlattice are in the metal between this single plane of large bubbles and the specimen surfaces and also in the metal columns between neighbouring large bubbles (see Fig. 4). The two bubble lattices thus coexist in the atomic lattice of the host metal. The single plane of large bubbles may be a remnant of a fully ordered three-dimensional bubble macrolattice present in the target before thinning.

The discovery of a bubble macrolattice increases the experimental knowledge with which the various theories of bubble ordering should be consistent. There are three classes of such theories involving: (1) elastic interaction between bubbles[22-24], (2) planar diffusion of host interstitial atoms[25,26] and, (3) interaction between dislocations 'punched out' from overpressured bubbles[9,27-29]. Although these mechanisms are complex and each of the theories has both advantages and disadvantages (see, for

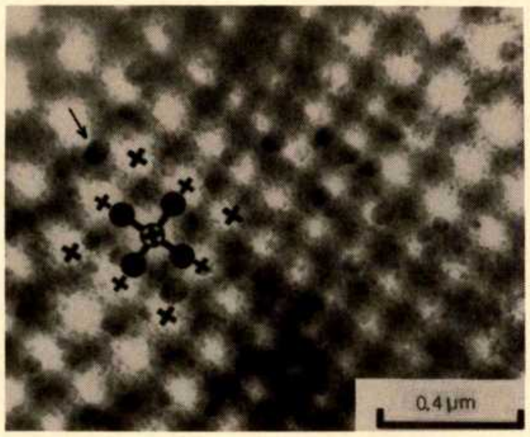

FIG. 3 A direct transmission electron micrograph showing patches in which all the bubble-like artefacts are larger than in the surrounding regions. Except for such isolated patches the size and spacing of artefacts is remarkably uniform throughout the specimen with typical diameters of ~70 nm and nearest-neighbour separations of ~105 nm (measured along ⟨220⟩). The grid (solid black circles and lines) shows the typical spacing of artefacts in the surrounding regions. The correlated growth of artefacts in a patch forms part of the evidence that the artefacts are helium bubbles. The disk-like images, such as that indicated by the arrow, are attributed to pieces of metal blown out from the surface by the bursting of large bubbles during ion-beam thinning.

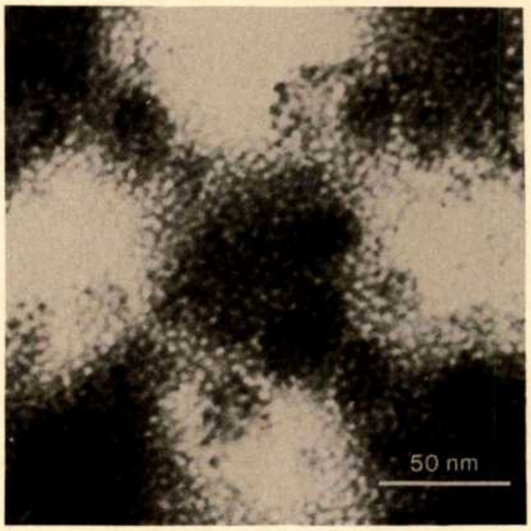

FIG. 4 A high-magnification transmission electron micrograph (under-focus contrast) showing the small bubbles (~2 nm in diameter) in the metal between the large bubbles that lie on the macrolattice. The four light areas are the positions of large bubbles that have intersected the specimen surface. The large bubbles have distorted the surrounding metal and perturbed the alignment of the bubble superlattice, but traces of bubble alignment can still be seen. The ordering of the small bubbles can be made much more obvious by carefully removing the remnants of the large bubbles by electropolishing.

example, ref. 30) precluding a detailed analysis here, some preliminary comments can be made.

The ratio of lattice parameter to bubble radius for the macrolattice is about four. This value is in agreement with treatments[22,23] based on the ordering of spherical cavities owing to elastic interactions. It is not yet clear, however, that elastic interactions are sufficiently strong to explain bubble ordering[30]. Despite imperfections, such as macrolattice 'dislocations' formed by terminating bubble rows, there is a clear long-range character to the ordering in the plane of the implanted layer. There seems to be no ready explanation for this feature in terms of the theories based on the planar diffusion of metal atoms. For example, it seems improbable that the mean free path for the lateral diffusion of gold atoms in a metal matrix containing high levels of gas and damage could be large enough to explain the long-range ordering.

The dislocation-punching theories of bubble ordering are based on the assumption that there is a strong mutual repulsion acting between neighbouring bubbles. This repulsion is in addition to the attractions and repulsions proposed in earlier theories[21,22]. The mutual repulsion was attributed to a repulsion between dislocation loops of diameter comparable with that of the bubbles. (Here, a dislocation loop can be thought of as a circular planar disk of atoms, one atom thick, pushed out from the surface of the bubble into an interstitial position between adjacent dense-packed atom planes of the host metal.) These loops were postulated to lie in the metal separating nearest-neighbour bubbles[28]. In the case of the superlattice such dislocations have never been observed. This led to the proposal that the mutual repulsion might result from strong compressional stresses set up in the metal between bubbles by the tendency of overpressured bubbles to punch dislocations rather than from the presence of dislocations[9–11]. It should be possible to image dislocations of a size comparable with the diameter of the large bubbles forming the macrolattice but we have found no evidence of such dislocations. This supports the proposal that the interaction between bubbles may need to be modelled, not in terms of existing dislocations, but rather in terms of the compressional stress in the metal set up between neighbouring bubbles[9–11]. □

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New pressure-induced transformations of silica at room temperature

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SILICA has many polymorphs, and the solid-state transformations between these under pressure have been studied extensively to gain insight into the mechanisms of such high-pressure transitions in general. Most of the transformations of silica polymorphs have been thought to require high temperatures as well as high pressures. Recently, however, a pressure-induced change in silicon coordination in silica glass[1] and amorphization of quartz and coesite (a high-pressure phase)[2] have both been observed at room temperature. Here we report two new room-temperature pressure-induced transformations of silica, observed by *in situ* X-ray diffraction. At pressures above 10 GPa, cristobalite transforms into a new phase, and above 30 GPa a second new phase is formed, both of which are crystalline. Above 60 GPa, however, quartz transforms to poorly crystallized stishovite, probably via an amorphous state. The new high-pressure polymorphs are probably metastable, and may involve a change in silicon coordination.

The crystal chemistry of silicate melts and glasses indicates that pressure-induced reversible changes between four-fold and six-fold Si coordination may occur under pressure[3]. Such changes in coordination affect the melt and glass density and viscosity, and could enhance the processes of chemical differentiation with depth in the Earth's mantle[4]. Cristobalite is a high-temperature polymorph of SiO_2 with a very loose structure. A molecular dynamics calculation predicted[5] that at 16.5 GPa cristobalite may transform into a metastable structure of space group *Cmcm*, containing both four-fold and six-fold Si coordination. This phase was predicted to transform into stishovite at 23 GPa. Our experiments were performed to test these predictions. The details of these experiments are very similar to our previous study on SiO_2 up to 120 GPa (ref. 6). To avoid the effect of moisture, finely powdered samples of synthetic cristobalite and quartz were dried at 100 °C for 2 h and embedded in a small hole (100 μm) in a stainless-steel

TABLE 1 X-ray diffraction data for SiO_2

Phase	Pressure (GPa)	*d* spacing (Å)	Intensity
XI	15.1	3.098	S
		2.883	VW
		2.702	VS
		2.373	M
		2.180	W
		2.026	M
XII	53.1	2.493	W
		2.114	VW
		1.917	M
		1.859	M
XIII	0.0*	3.166	W
		2.966	VW
		2.595	S
		2.244	VW
		1.972	W
		1.933	W
		1.834	VW
		1.494	M

Starting material was cristobalite. S, strong; M, medium; W, weak; VS, very strong; VW, very weak.

* Quench product recovered from 53.1 GPa.

gasket. Pressure in the sample chamber was measured using ruby fluorescence[7]. Three or four ruby chips were spread in the sample chamber and concentric distributions of pressure were observed. Average pressures as well as maximum and minimum pressures in the area irradiated by X-rays (70 μm across) were used for the analysis. The *in situ* X-ray observations of the samples at high pressure and ambient temperature were made using a rotating-anode high-power X-ray source operated at 55 kV and 160 mA. Filtered Mo Kα radiation was collimated by a 60-μm pin hole and the diffracted X-rays were measured by a Debye-Scherrer camera with a radius of 57.3 mm. The typical exposure time was three days. X-ray diffraction observations were also made of recovered samples at ambient temperature and pressure. To determine the stable phase, samples mixed with a small amount of platinum black (which absorbs Nd-YAG laser radiation) were heated, at the desired pressure, by the laser radiation, and examined by *in situ* X-ray diffraction.

Results of the room-temperature compression of cristobalite are shown in Fig. 1. Because of its loose structure, cristobalite is much more compressible than the other polymorphs of silica. The zero-pressure bulk modulus, K_0, calculated from the X-ray diffraction data using a Birch-Murnaghan equation of state is 18 ± 1 GPa, very close to the value of 17.2 GPa obtained by molecular dynamics calculations[8]. This value is more than one order of magnitude smaller than that of stishovite. A similar bulk modulus, $K_0 = 15.7$ GPa, was obtained in another X-ray diffraction study[9].

At 10 GPa and room temperature, cristobalite transforms into a new phase XI that is different from stishovite. None of the known polymorphs of silica can account for the observed diffraction pattern (Table 1) and the structure of this new phase has not yet been determined. This phase is unquenchable and on release of pressure, it transforms back into cristobalite. Molecular dynamics simulations[5] have also predicted an unquenchable *Cmcm* phase, but the diffraction pattern of our high-pressure phase is different to that expected from the simulation phase, so it is not clear whether the two are related.

Further compression produces another new phase, XII. The X-ray diffraction lines of this phase are weak and broad, however, making analysis of the structure very difficult. This phase is also different from the known polymorphs of silica. On the release of pressure, it transforms into another new phase, XIII, different from cristobalite and other polymorphs of silica (Table 1). When the XI phase was heated above 1,000 °C at 15 GPa, it transformed into a single phase of stishovite. We therefore suggest that the new phases formed by compression of cristobalite at room temperature are metastable.

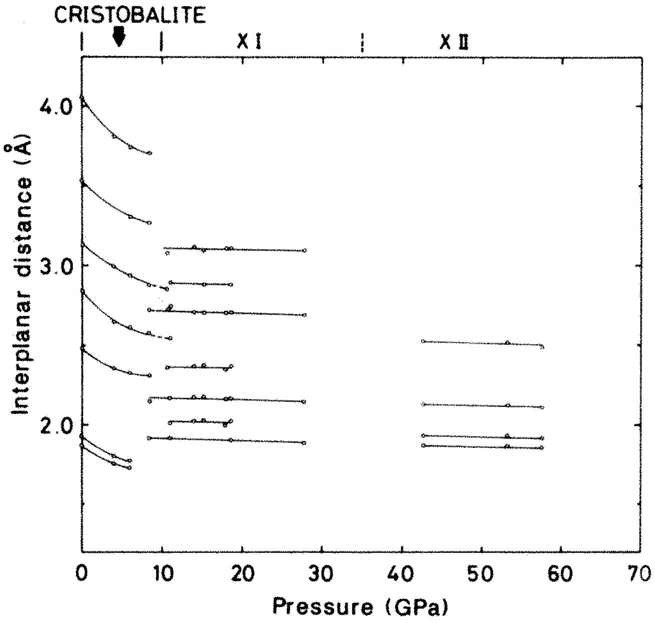

FIG. 1 Pressure dependence of interplanar spacing of cristobalite.

TABLE 2 Products of room-temperature compression of quartz

Run	407	421	725	702	921
Pressure (GPa)	59* (61-56)†	69 (72-65)	72 (74-69)	62 (66-56)	70 (74-67)
Time (h)	72	72	72	1	72
High-pressure phase	rutile	rutile	rutile	‡	‡
Recovered sample at ambient condition	‡	‡	rutile	amorphous	rutile

* Average value in irradiated area (70 μm across).
† Maximum and minimum pressures in irradiated area.
‡ Not measured.

The experimental results for quartz are summarized in Table 2. The transformation from quartz or coesite to stishovite had been believed to require temperatures >600 °C[10], but our results indicate that stishovite can be formed even at room temperature when enough excess pressure is applied. This stishovite is poorly crystallized and has very broad and weak diffraction lines. Moreover, the observed 111 diffraction line is about twice as intense as the 110 line. On the other hand, for randomly oriented particles, the 111 line is 35% less intense than the 110 line. This implies the existence of preferred orientation in the sample.

To investigate the kinetics of the quartz-stishovite phase transformation, two experiments were carried out (Runs 702 & 921 in table 2). Strong X-ray irradiation of crystals can cause dislocations, which might accelerate the crystallization or phase transformation. To avoid this, we did not use high-pressure *in situ* X-ray diffraction in these runs. We did not find any diffraction lines from a sample recovered after holding quartz at 62 GPa for 1 h (Run 702), but we did observe weak broad stishovite lines when the same starting material was kept at 70 GPa for 3 days (Run 921). The quartz-to-stishovite transition at room temperature is therefore very sluggish.

Our results indicate that the room-temperature pressure-induced transformation from quartz to stishovite occurs through the amorphous state. This implies that the activation energy for the formation of silica polymorphs decreases dramatically under very high pressure, as well as in the low-pressure region[11]. Williams and Jeanloz[1] suggested that a pressure-induced change in the coordination of silicon occurs at room temperature, based on the observation of an infrared absorption of silica glass. Our crystallographic evidence demonstrates that this kind of transformation can occur at room temperature. Our results are also consistent with the model proposed by Stolper and Ahrens[3], which suggested a coordination change of silicon by displacement of the SiO_4 tetrahedron, without a large diffusion of atoms.

Our high-pressure experiments were performed by directly compressing powdered samples between anvils and large pressure gradients in the sample chamber were observed (Table 2). It is therefore difficult to discuss the details of the mechanism of transformation. To avoid this problem, a quasi-hydrostatic pressure-transmitting medium, such as solidified rare gas, could be used.

Cristobalite does not show pressure-induced amorphization but transforms directly into the crystalline phase. The second high-pressure phase, XII, is stable at ~60 GPa, whereas stishovite is formed when quartz is used as the starting material. The structure of XII has not yet been determined but we have estimated that its compressibility is very small. The value of d/d_0 for each diffraction line of XII is between 4.3×10^{-4} GPa^{-1} and 8.7×10^{-4} GPa^{-1} at ~50 GPa, almost the same as that of stishovite (the average value for the four diffraction lines is ~6.8×10^{-4} GPa^{-1})[6]. The X-ray pattern of XII phase has no diffraction line corresponding to $d > 3.0$ Å. These results imply a close-packed structure.

To understand the difference between the transformations of quartz and cristobalite, we need to know the Si–O coordination number of the XII phase. Both XI and XII phases are poorly crystalized, however, and the quality of the diffraction patterns are not sufficient to solve the structures. Structural information from Raman scattering, infrared absorption and EXAFS (extended X-ray absorption fine structure) would be useful. □

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A 210,000-year record of barium variability in the deep northwest Atlantic Ocean

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THE deep ocean is the storehouse for most of the carbon and nutrients in the ocean–atmosphere system; together with its outcrops at high latitudes, the deep ocean is of great importance in driving glacial–interglacial changes in the carbon dioxide content of the atmosphere[1–3]. The chemical and physical structure of the deep glacial oceans can be reconstructed by means of palaeochemical tracers; for example, the cadmium and carbon isotope contents of benthic foraminifera have been used to reconstruct the phosphate (or labile nutrient) compositions of deep waters[4–14]. Recently, it was proposed[15] that barium in benthic foraminifera could be used to reconstruct the distribution of refractory, deep-regenerated chemical properties in the water masses of the glacial oceans. A reconstruction of barium concentrations in the oceans at the time of the Last Glacial Maximum revealed the greatest changes in the North Atlantic, where barium concentrations in deep water were up to 50% higher than at present[16]. Here we present a record of the benthic Ba/Ca ratio in the deep northwest Atlantic stretching back to interglacial stage 7 (~210 kyr ago), encompassing two full glacial cycles. A comparison of the barium record with that of the other nutrient-like tracers lends confidence in the general agreement between these tracers while showing some spectral variability unique to barium, thus underscoring its utility as an additional palaeoceanographic tracer.

Piston core CHAIN 82, station 24, core 4PC was raised from 3,427 m on the western flank of the Mid-Atlantic Ridge (41°43′ N, 32°51′ W). The sedimentation rate averages ~3 cm kyr^{-1}, although the rate is not uniform over the length of the core. The sample depths reported here are for contiguous samples relative to the core liner; there was a void interval between 347 and 380 cm apparently resulting from mechanical fracturing[5].

Figure 1 illustrates the benthic foraminiferal Ba/Ca record, as well as oxygen isotope, carbon isotope and Cd/Ca records from a previous study[5]. Ba was determined on purified shells by isotope-dilution plasma mass spectrometry[15,16]. The analytical precision of the Ba/Ca ratios is estimated to be better than 3%. Many of the samples were run in duplicate, as indicated in Table 1. Ba/Ca ratios were determined on samples of the benthic foraminifera *Uvigerina spp.* where available. Certain intervals have low to near zero *Uvigerina spp.* abundances: 0–35 cm (stage 1), 250–300 cm (stage 5a), 450–465 (stage 5e) and 650–670 cm (stage 7a–b)[5]. *Cibicidoides kullenbergi* was used instead of *Uvigerina spp.* between 0 and 30 cm. Calibration of these two species for uptake of Ba indicates no statistically significant difference[15]. Ba/Ca ratios from *Uvigerina spp.* were used in the other low-abundance intervals; these values should be interpreted with caution because small samples were used for analysis. In addition, bioturbation can bias any foraminiferal parameter measured in zones of low abundance.

The timescale for core 4PC shown in Fig. 1 is based on the accepted timing for the main features of the oxygen isotope record identifiable in this core[5,17]. The bottom of the core (668 cm) has been assigned to the transition between stage 7a and 7b, or ~220 kyr ago. This produces a realistic sedimentation rate for stage 7 (~2 cm kyr^{-1}) and is justifiable given that the benthic oxygen isotope record never reaches the heavy values (~4‰) characteristic of stage 7b. In addition, the disappearance of *Uvigerina spp.* in the bottom 20 cm of the core is consistent with the change observed in early stage 7a in neighbouring core V30-97[18].

The principal features of the Ba/Ca record show a strong covariance with the generally accepted climate cycles of the past 200,000 years[19,20]. In general, minima in benthic Ba are associated with interglacial stages and maxima are associated with glacial stages. Interglacial stages (1, 3, 5 and 7) are characterized by Ba/Ca values of ~1.8–2.6 μmol mol^{-1}, and glacial stages (2, 4 and 6) are characterized by values of ~2.6–4 μmol mol^{-1}. The most Ba-depleted deep waters at these sites were present in stages 5a, 5c and 7, and the most Ba-enriched deep waters were present during stage 2 and 6. One might also expect stage 5e to be very low in Ba and measured values in this interval are probably not representative of the true values because of extremely low *Uvigerina* abundances[5].

The Ba and Cd records from core 4PC are generally similar in confirming high nutrient levels during glacial periods and low nutrient levels during interglacial periods (Fig. 1). There are, however, clear differences between the two records: (1) as previously noted from a study of both Atlantic and Pacific cores[16], the Ba contrast between the oceans vanishes during glacial stage 2. The high Ba values of stage 6 of core 4PC suggest the same for other glacial extrema, although this remains to be proven; (2) little direct correspondence is seen between the short-timescale fluctuations of Cd and Ba. Comparison of the Ba record with the carbon isotope record reveals that Ba/Ca maxima and δ^{13}C minima correspond in stage 2, 4, 5d and in the three cycles of stage 6. A direct comparison of the carbon isotope and Ba data is misleading, however, because a large portion of ^{13}C variability is due to transfer of carbon between the ocean and continental organic carbon reservoir, which is not expected to affect Ba[21,22].

The short residence time of barium in the ocean, estimated at ~10,000 years[23], is especially important in any consideration of its long-term variability. A survey of Ba in the oceans of the Last Glacial Maximum suggest that average Ba concentration of the ocean might have been ~15% lower[16]. On the 200,000-year timescale of the core 4PC record, changes in the Ba content of the oceans are quite possible. The variability recorded by the benthic foraminifera from core 4PC argues against large changes

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TABLE 1 Ba/Ca in benthic foraminifera of core 4PC

Interval (cm)	Ba/Ca (μmol mol^{-1})	SD	*n*	*r*	Interval (cm)	Ba/Ca (μmol mol^{-1})	SD	*n*	*r*	Interval (cm)	Ba/Ca (μmol mol^{-1})	SD	*n*	*r*
0–2	2.33*	0.06	2		224–226.5	2.88	0.04	2		466–468	2.95	0.01	2	
4–5	2.24*	0.01	2		226.5–228.5	2.87	0.07	3		468–470	2.83	0.07	2	
8–9	2.26*		1		228.5–230	3.19		1	1	470–471	2.98		1	
12–13	2.42*	0.08	2		230–233	3.00		1		472.5–475.5	3.23	0.10	2	
19.5–20.5	2.40*		1		233–236	2.65	0.20	3		478.5–482	3.91	0.24	2	
22–24	2.38*		1		239–242	2.30	0.20	3		485.5–489	4.17		1	
25–26	2.72*		1		241.5–243	1.96		1		490–492	3.74	0.09	2	
35–36	2.51		1		243–246	1.82	0.04	2		492–494	3.54		1	
37–38	2.73		1		247–252	1.95	0.07	2		494–497	3.70		1	
48–49	2.81		1		254–256	2.61	0.04	2		497–500	3.42		1	
53.5–54.5	3.16		1		255–264	2.55		1		499–501	3.89	0.28	2	
56–59	3.41		1		266–271	2.37		1		502–504	2.92		1	
59–62	3.54	0.02	2	1	272–279	3.03		1		504–507	3.01		1	
67–69	3.49	0.08	3		286–291	2.82		1		510–513	3.02		1	
70–72	3.54		1		291–295	2.56		1		515–517	2.67		1	
73–75	3.14		1		295–298	2.49		1		517–519	2.63		1	
77–79	3.33		1		298–300	2.64		1		519–522	2.89	0.04	2	
84–87	3.50		1		300–303	2.40		1		523–525	2.68		1	
89–90	3.46		1		303–306	2.40		1		526–529	2.62	0.13	2	
93–95	2.92	0.04	2		307–309	2.34		1		529.5–532	3.18	0.08	2	
95–97	2.97	0.29	2		310–312	2.29		1		535–537	2.74	0.06	2	
100–103	2.71		1		312–316	2.54		1		537–539	4.53	0.38	2	
104–107	3.00		1		320–322	2.12		1		539–541	3.51	0.47	3	
109–111	2.73		1		322–324	2.17		1		541–544	3.08	0.16	3	2
114–116	2.56	0.22	3	1	325–328	2.18		1		544–547	3.53	0.06	2	
116.5–119	3.52	0.22	2		328–330	2.10		1		544–547	3.48		1	
119–122	2.53		1		330–332	2.25		1		547–550	3.57	0.31	3	
119–126	2.75		1		333–334	2.13		1		550–553	2.62	0.09	2	
123–126	2.52		1		334–336	2.09		1		553–556	2.31	0.16	2	
128–130	2.92		1		336–338	2.00		1		560–563	2.41	0.15	2	
130–133	2.90		1		339–341	2.09	0.01	2		563–565.5	2.82	0.49	4	
133–136	3.37		1	1	341–343	1.92		1		566–568.5	2.43	0.24	2	
136–138.5	2.57		1		343–345	1.86		1		568–571	2.47		1	
138.5–141	2.80		1	1	345–347	2.05	0.02	2		571–573.5	2.69	0.07	2	
141–143.5	2.53	0.01	2		347–348	1.95		1		573.5–575.5	2.55		1	
143.5–146	2.38	0.09	2		381–383	1.98		1		575.5–578.5	2.75	0.12	3	
146–148	2.40		1		384.5–7.5	2.25		1		578.5–581	2.55	0.11	2	
148–150.5	2.28		1		384.5–387.5	1.89		1		581–584	2.73	0.07	2	
150.5–153	2.39		1		384.5–387.5	2.05		1		584–587	3.27	0.06	2	
153–155	2.54	0.06	2		393–395	2.14		1		587–590	3.12	0.03	2	
155–157.5	2.93		1		395–397	2.40		1		590–593	3.36		1	
157.5–159	2.96	0.20	2		398–400	2.20		1		593–595	3.16		1	
159.5–162	3.17	0.32	2		400–402	2.29	0.19	2		596–598	2.98		1	
162–164.5	2.72	0.02	2		402–404	2.23	0.05	2	1	598–600	3.32	0.03	2	
164.5–167	2.54	0.12	2	1	404.5–407	3.04	0.29	4		600–603	3.34	0.27	2	
167–170	2.95	0.01	2		410–413	3.48		1		604–606	2.87	0.01	2	
170–172.5	2.61		1		413–415.5	2.17		1		607–610	2.35	0.04	2	
172.5–175	2.53	0.32	2		415.5–418	2.21	0.17	2		610–612.5	2.23	0.00	2	
177–179	2.15	0.03	2		418–420.5	2.35	0.09	2		613–616.5	2.26		1	
179.5–181.5	2.16		1		421–424	3.79	0.25	2		616.5–619.5	2.20		1	
185–187	2.34		1		423–426	2.45		1		620–623	2.04		1	
187–190	2.25		1		426–429	2.42	0.27	3	1	623–626	2.10	0.02	2	
190–193	2.29		1		429–432	2.31		1		627–630	1.86		1	
193–195	2.46		1		432–435	2.84	0.74	3		630–633	1.99		1	
195–198	2.47		1		435–438	2.75	0.25	2		633–636	1.92	0.18	2	
198–201	2.70		1		438–440	2.46	0.29	3		636–638	1.70		1	
201–203	2.74	0.04	2		440–442	2.37	0.11	3		638–640	2.04	0.29	2	
203–205	2.68		1		442–444	2.33		1		640–642	2.11	0.05	2	
207–209	2.70	0.16	2		444–446	2.24		1		642–644	2.01		1	
210–214	2.58	0.07	2		446–450	2.20		1		644–646	2.20		1	
214–217	2.80	0.20	2		456–458	2.35		1		646–648	2.00	0.10	2	
217–220	2.92	0.06	2		458–462	2.72		1		651–657	1.96		1	
220–222	2.93	0.38	2		464–466	3.23		1						

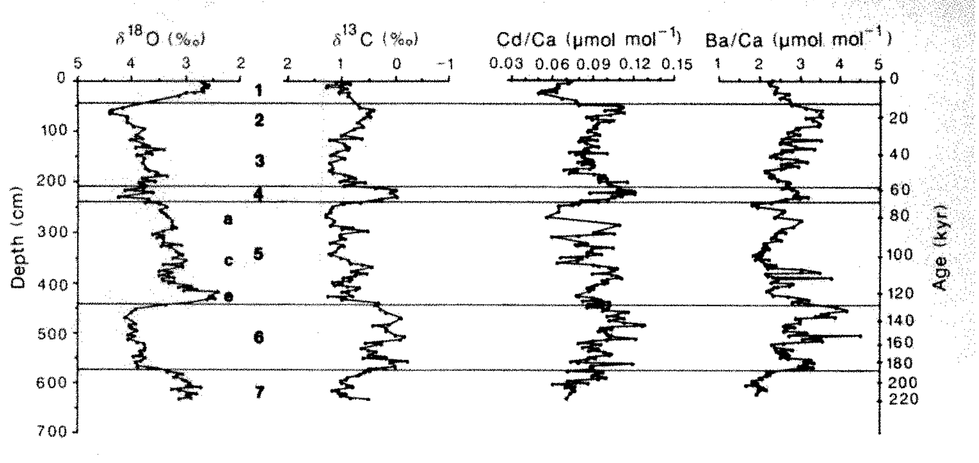
For depths with 2 replicates, standard deviations (SD) are differences from the mean. *n* is the number of replicates included in the mean and *r* is the number of replicates rejected from the mean.

* *C. kullenbergi.*

in the Ba content of the oceans over the past 200 kyr, however, because the interglacial minima are of similar magnitude between stages 1, 5 and 7. The question of change in oceanic Ba content cannot be answered adequately without a complementary Pacific record.

Two previous studies of core 4PC indicated that down-core variability in benthic foraminiferal Cd (and some of the down-core variability in $\delta^{13}C$) can be explained by variations in the flux of nutrient-depleted water to this site[5,22]. Such variations would affect Ba in a similar manner because warm surface ocean

FIG. 1 Ba/Ca of benthic foraminifera (mostly *Uvigerina spp.*; see Table 1) plotted against depth for core 4PC. Also illustrated are oxygen isotope (*C. wuellerstorfi*), carbon isotope (*C. wuellerstorfi*) and Cd/Ca (mostly *Uvigerina spp.*) data for the same core[5]. Stage boundaries are based on the oxygen isotope record. Depths below 348 cm have been corrected by −32 cm because of a core void between 348 and 380 cm. The age model plotted on the right-hand side of the figure is based on correlation of the oxygen isotope record with a stacked oxygen isotope record[17]. ($\delta^{18}O$ (‰) $=\{[(^{18}O/^{16}O)_{sample}/(^{18}O/^{16}O)_{standard}]-1\}\times 1{,}000$, where standard is PDB (NBS20); $\delta^{13}C$ is defined similarly, standard is PDB.)



waters are depleted in Ba. The fact that records of all three tracers indicate higher nutrients in Atlantic deep waters in glacial times (during which there is reduced flushing) suggests that at least some of the temporal variability in each tracer is the result of variations in the thermohaline circulation of the North Atlantic.

Spectral analysis has been applied to our Ba data and to the Cd, carbon and oxygen isotope data[5,22] to evaluate the frequency response of these records with respect to the Earth's orbital parameters. The technique is based on the fast Fourier transform (FFT) method and is described in ref. 22.

Figure 2 illustrates periodogram and log power spectra from the benthic foraminiferal Ba record of core 4PC. The strongest power is found for frequencies of 0.0094 (106 kyr), 0.0189 (53 kyr) and 0.0425 (23.6 kyr). The power at 100 kyr is seen in almost all palaeoclimate records and corresponds to the period of eccentricity in the Earth's orbit. The power at 106 kyr in the Ba record accounts for ~22% of the total variance (area under the peak). The power at 23.6 kyr corresponds to the period of precession in the Earth's orbit. The peak at 23.6 kyr is the dominant peak for Ba, accounting for ~34% of the total variance in the spectra. The occurrence and magnitude of the peak at 53 kyr (24% of the total variance) is surprising[24-26]. Although this periodicity corresponds to the beat frequency between obliquity and precession, it is not a component frequency of insolation forcing. (A similar situation exists for the eccentricity modulation of precession and the 100-kyr climate cycle.) The 53-kyr component of the Ba record is approximately in phase with the beat between obliquity and precession in the Northern Hemisphere. This observation needs to be confirmed by other long records before speculating about its cause.

The dominance of the 23-kyr precessional cycle in the Ba spectra stands out as a key factor that distinguishes the Ba record from that of Cd and carbon isotopes. Spectral analysis of August sea surface temperature in nearby core V30-97 indicates dominance of precessional power similar to that observed in the Ba spectra from core 4PC[18]. As Cd, Ba and carbon isotopes all potentially record variations in the flux of nutrient-depleted waters to the deep Atlantic, this difference suggests that either Ba is more sensitive than Cd or carbon in recording precessional forcing of deep-water formation or, that variability in these three tracers is forced by more than one mechanism. The first hypothesis seems less likely because Cd, which is more depleted in warm surface waters than Ba (that is, has a greater dynamic range), should always be more sensitive to variations in the flux of nutrient-depleted surface water. The second hypothesis is intriguing because it suggests that some of the temporal change in Ba might reflect a process that does not leave a prominent imprint on the records of Cd and carbon isotopes. This hypothesized process or mechanism must be strongly forced by the 23-kyr precessional cycle. A model developed in ref. 16 suggests that differences in the distribution of Ba and Cd (and carbon isotopes) in the oceans of the Last Glacial Maximum might be

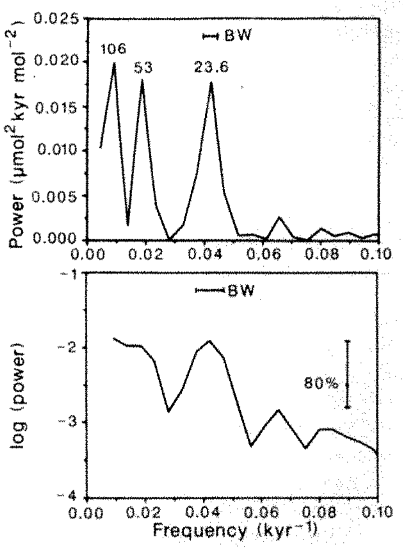


FIG. 2 Periodogram and log power spectra for the core 4PC Ba record. Bandwidths (BW) and 80% confidence intervals are indicated. The power spectra are based on two-band averaging. See ref. 22 for details of spectral analysis. The record is first interpolated to create a record of 256 equally spaced points. To minimize spurious spectral properties, the interpolated record is detrended (subtraction of the linear trendline) and tapered (the first and last 10% of the record are multiplied by a function that varies from 0 at the ends of the records to 1 at the inside 10%, following the shape of a half-bell-shaped cosine); these changes reduce the resolution and amplitude of spectra peaks slightly. This modified record is then transformed using the discrete fast Fourier transform algorithm, which transforms the 256 real data points in time to a series of 128 real (a_j) and imaginary (b_j) coefficients as a function of frequency (where $f=1/T, 2/T, \ldots, 128/T$, where T is the record length). The periodogram is the power ($a_j^2+b_j^2$) as a function of these frequencies. To improve the statistical properties of the periodogram, the power in adjacent frequency bands is averaged to provide a power spectrum. The 80% error bar indicates the amplitude of peaks exceeded only 20% of the time by a random noise signal. Although this method is different from the autocovariance technique also used to calculate spectra, the two methods are mathematically equivalent and no significant differences can exist between the methods when both are applied cautiously to avoid artefacts.

the result of the sensitivity of barium in the deep Atlantic to enhanced upwelling and the subsequently increased particulate Ba fluxes in the glacial Atlantic. There is some evidence to tie precessional forcing to enhanced equatorial upwelling in the Atlantic; when perihelion is aligned with boreal winter the zonal velocity of the trade winds is believed to be stronger and the equatorial Atlantic experiences maximum divergence and increased productivity[27,28]. Presumably, particulate Ba flux out of the euphotic zone is greatest during these periods[29-31], which would reinforce a coincidence of Ba maxima with minimum insolation during boreal summer (aphelion). A test of this hypothesis is to examine the phase relationship between faunal temperature indices for the equatorial Atlantic[28] and the Ba record. Such a comparison suggests that high barium in North Atlantic deep water lags sea surface temperatures of the cold equatorial Atlantic by up to 2-4 kyr. Although this lag is barely within the resolution of the data, it could be explained by vector addition of equatorial upwelling and deep-water formation (4-kyr lag relative to sea surface temperature of the equatorial Atlantic). □

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The role of hydrogen in the electrical conductivity of the upper mantle

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INTERPRETATIONS of the observed electrical conductivity of the upper mantle have relied on laboratory studies of dry olivine and basaltic melts[1,2]. These studies indicate that the conductivity of the asthenosphere is higher than that of olivine, suggesting that partial melting or other high-conductivity mechanisms such as grain-boundary phases are required[2,3]. Recent experimental studies[4-6], however, have demonstrated that various kinetic processes in silicate minerals are greatly enhanced by the dissolution of hydrogen. As hydrogen is probably present in the upper mantle, studies on dry olivine may not therefore be relevant to the real Earth. Here I use experimental data on the solubility and diffusivity of hydrogen in olivine[5,7] to estimate its effect on the electrical conductivity. I conclude that the observed high conductivity in the asthenosphere can be attributed to solid-state conductivity in olivine if a small amount of hydrogen is present. Partial melting of the asthenosphere on a global scale is therefore not required.

The electrical conductivity of rocks depends on several factors including temperature, pressure, degree of partial melting and the chemical characteristics of the environment (such as oxygen fugacity)[1,2,8]. Experimental studies on dry (water- or hydrogen-free) olivine suggest that implausibly high temperatures ($T > 1{,}500\,^{\circ}\mathrm{C}$) are required in the asthenosphere to explain the high conductivity observed ($\sim 0.1\text{-}0.01\ \mathrm{S\,m^{-1}}$)[1,2,9,10], if solid-state conduction through bulk olivine crystals is the assumed mechanism. These observations have been interpreted as providing strong evidence for the existence of partial melt or of other mechanisms for high conductivity[2,3].

Recent experimental studies have demonstrated, however, that solid-state transport in silicates is made considerably more facile by the dissolution of a small amount of hydrogen[4-7]. Infrared spectra of olivine annealed in a hydrous environment show absorption peaks due to the OH stretching vibration[5-7,11,12]. These observations indicate that hydrogen (or water) is incorporated in olivine as a charged species, and so should increase the electrical conductivity if its solubility and mobility are large enough. Thus studies on dry olivine may not be relevant to the real Earth if some hydrogen or water is present in the upper mantle[13]. The electrical conductivity of olivine in the presence of hydrogen or water has been measured experimentally only at atmospheric pressure[14]; it is difficult to extrapolate these data as the solubility of hydrogen probably increases significantly with pressure. It is possible, however, to estimate theoretically the effect of hydrogen on conductivity once its concentration and mobility in olivine are known.

The contribution of charged, mobile species to the electrical conductivity of a solid is given by the Nernst-Einstein relation[15]

$$\sigma = fDcq^2/kT$$

where σ is the electrical conductivity, f is a numerical (correlation) factor approximately equal to unity, D is the diffusivity, c is the concentration, q is the electrical charge of the charged species, k is the Boltzmann constant and T is the temperature. The validity of the Nernst-Einstein relation for dry olivine has been demonstrated in refs 16 and 17, where it was suggested that electrical conduction in dry olivine at high temperatures ($T > 1{,}200\,^{\circ}\mathrm{C}$) is due to diffusion of Mg^{2+} (or Fe^{2+}), although at lower temperatures the charge carriers are holes[8,16].

I have used the Nernst-Einstein relation to calculate the electrical conductivity in olivine containing hydrogen (Fig. 1). I assume that the hydrogen-related charge carriers have a charge $q = +e$ and I use the values of diffusivity determined experimentally[7] at a pressure $P = 300$ MPa. Because of the uncertainties in the hydrogen content of the upper mantle, the conductivity was calculated for several values of this quantity. Experimental studies[7] show that the solubility of hydrogen at $P = 300$ MPa is

20-80 p.p.m. H/Si, and both theoretical and experimental studies suggest that the solubility of hydrogen increases with pressure[5,6,19]. At asthenospheric pressures (~3-5 GPa), I therefore assumed values of 100 and 1,000 p.p.m. H/Si. The calculations indicate that the electrical conductivity of olivine increases significantly when hydrogen is present (Fig. 1). The conductivity at a realistic asthenospheric temperature of $T \approx 1{,}300$ °C (ref. 18) is close to that estimated from geomagnetic sounding (~0.1-0.01 S m^{-1})[9,10].

Some simplifying assumptions have been made in this calculation. First, no correction was made for the pressure-dependence of hydrogen diffusivity, for which no experimental results are available. This assumption is probably justified in view of the observed small activation energy for hydrogen diffusion, which implies a small activation volume. Second, the solubility of hydrogen may depend on oxygen partial pressure (and oxide activity)[7,19,20]. This effect is, however, relatively minor. Moreover, as the oxygen fugacity in the upper mantle is not well constrained, I have not considered its effect in detail. Third, the experimental data on hydrogen diffusivity were obtained at temperatures of 800-1,000 °C, and extrapolation to higher temperatures was made assuming the same activation energy. This extrapolation probably gives a lower bound for the conductivity at higher temperatures, because activation energies often increase with increasing temperatures (Fig. 1).

The results in Fig. 1 were measured along the axis of highest conductivity. Because the hydrogen diffusivity is anisotropic[7], the actual conductivity of olivine aggregates will be somewhat lower because of the spread in orientations.

I have assumed that the predominant charge carriers are protons. Another possibility is that the dissolution of hydrogen increases the concentration and/or mobility of other charged species (such as Mg^{2+} or holes). Karato *et al.*[6] found that creep controlled by Mg^{2+} (or Fe^{2+}) diffusion, and thus the diffusion of the ions themselves, is enhanced in the presence of hydrogen. But as the details of this effect are not known, I have not considered it here.

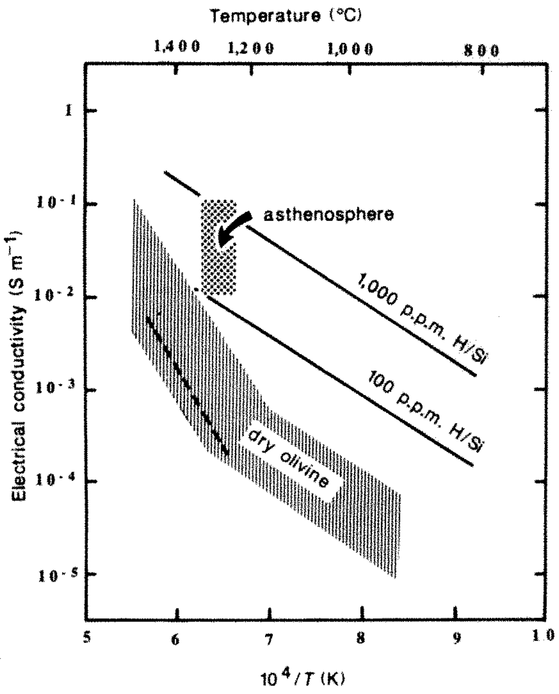

FIG. 1 Electrical conductivity in olivine as a function of temperature and hydrogen content. Data for dry olivine correspond to the hatched region[1,8]. The dashed line shows the electrical conductivity calculated from the diffusion coefficients of Mg^{2+} (or Fe^{2+}) under dry conditions. Solid lines show the electrical conductivity calculated from experimental[7] diffusivity of hydrogen. The dotted region indicates the range of conductivity estimated from geomagnetic soundings and the estimated temperature of the asthenosphere. This region coincides with the estimated conductivity for a hydrogen content of ~200-2,000 p.p.m. H/Si.

These uncertainties make it difficult to quantify the effect of hydrogen on the electrical conductivity of olivine; but the qualitative conclusion that the conductivity is enhanced seems sound. This conclusion could be tested readily by experiments at high confining pressures. By comparing calculated values of the conductivity with geophysical observations, one can obtain an estimate of the hydrogen content in olivine in the asthenosphere (Fig. 1). For a conductivity of 0.1-0.01 S m^{-1} at a temperature of 1,300 °C, the hydrogen content is 200-2,000 p.p.m. H/Si. These values, however, depend on the absolute value of conductivity in the asthenosphere, which is somewhat uncertain because it depends on the (unknown) thickness of the high-conductivity layer[9,10]. Also, these estimates assume that other mechanisms for high conductivity, such as the presence of grain-boundary phases, do not operate.

This estimate of hydrogen content is higher than that determined[7] at $P = 300$ MPa but is reasonable when one takes into account the enhancement expected at higher pressures. No detailed experimental studies of this pressure enhancement have been made, but comparison of creep experiments[6] carried out for different water fugacities suggests that the solubility of water- or hydrogen-related defects increases with pressure as P^r, with $r \approx 1/3$-$1/5$. This implies that the solubility at asthenospheric pressures (3-5 GPa) is higher than at 300 MPa by a factor of two to three, which is consistent with the range of solubilities estimated above.

Significant enhancement of the solid-state kinetics is possible for water (hydrogen) contents much lower than that required to reduce significantly the solidus temperature and thus change the chemical composition of the melt (present at ~0.1 wt% or higher)[21]. In other words, hydrogen could enhance the upper-mantle conductivity without causing extensive partial melting in much of the asthenosphere. Indeed, the latter seems not to occur: the chemical composition of mid-ocean-ridge basalt is consistent with the partial melting of 'dry' (in the petrological sense, that is, water content less than ~0.1 wt%) peridotite[22], implying that partial melting of the oceanic upper mantle is limited to the region near oceanic ridges. Given the results of recent experimental studies on anelasticity (ref. 23, and I. Jackson and M. S. Paterson, manuscript submitted, *Nature*), it seems that all of the geophysical anomalies in the asthenosphere can be attributed to solid-state processes. ☐

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Chaotic, subduction-like downflows in a spherical model of convection in the Earth's mantle

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NUMERICAL models of three-dimensional, nonlinear, thermal convection of very viscous fluids have recently been developed in rectangular[1–4] and spherical[5–12] geometries as analogues of convection in the Earth's mantle. Here we describe model calculations for a compressible fluid in a three-dimensional spherical shell with 80% of the surface heat flow generated within the model mantle, in agreement with estimates for the Earth's mantle[13]. The Rayleigh number and the numerical resolution for these calculations are greater than those of our previous studies[9,10] of internally heated convection in a spherical shell. Our numerical solutions are strongly chaotic, with surface planforms dominated by long curvilinear downflows reminiscent of the descending slabs in the Earth's mantle. Although analogy to the Earth's mantle is necessarily imperfect, owing to, for example, the absence of variable viscosity and rheology—and hence of lithospheric plates—our results suggest that descending slabs play an important part in driving mantle convection, and that their chaotic evolution may influence the spatial and temporal behaviour of plates and thus the dispersal and aggregation of continents.

The numerical model[7] solves the nonlinear equations of motion that describe thermal convection of a newtonian (linear viscosity), infinite Prandtl number (highly viscous), anelastic compressible (no sound waves) fluid in a three-dimensional spherical shell for conditions that approximate the Earth's mantle. The thermodynamic variables and the three components of velocity are expanded in spherical harmonics (up to degree and order 85) to resolve their horizontal structures and in Chebyshev polynomials (up to degree 30) to resolve their radial structures. The nonlinear advection and viscous heating terms are calculated each timestep in grid space on 256 Fourier longitudinal levels, 128 Legendre colatitudinal levels and 33 Chebyshev radial levels. Our numerical solution is adequately resolved because the kinetic and thermal energies drop several orders of magnitude as functions of the horizontal and radial wave numbers and because the solution is in good agreement with a solution at a lower numerical resolution (spherical harmonic degree and order up to 63).

Our model lacks several important features required of fully realistic numerical simulations of mantle convection in the Earth. One is a viscosity that depends on temperature, pressure and the state of stress. Another is a variable rheology simulating the behaviour of tectonic plates. Phase and chemical transitions in the mantle also need to be modelled. Although these features will certainly affect the spatial and temporal details of the convection, we first need to learn what our present model reveals about the basic properties of time-dependent three-dimensional compressible convection in a spherical shell.

We examine a case that is partly heated within the model mantle (10^{-14} W kg^{-1}) and partly heated from the underlying core. An adiabatic polytrope with an index of 0.4 and a Grüneisen parameter of 1.1 determines the profiles of the reference-state pressure, density and temperature that approximate the Earth's mantle and about which the thermodynamic variables are perturbed. Impermeable stress-free boundary conditions are imposed on the velocity. The model temperatures are set to 3,270 K at the inner (core/mantle) boundary and 1,070 K at the outer boundary. Of the total temperature drop across the model mantle, 1,200 K is due to adiabatic compression and the rest, 1,000 K, is the superadiabatic temperature drop, which drives the convection. We specify a constant dynamic viscosity of 5.6×10^{22} kg m^{-1} s^{-1} and a constant thermal conductivity of 23 W m^{-1} K^{-1}. Owing to computing limitations, the thermal conductivity adopted in the model is about five times larger than the conductivity of actual mantle rocks. The volume-averaged Rayleigh number Ra (a measure of the convective vigour[7]) due to both the superadiabatic temperature drop and the internal heating is 1.6×10^6. This is ~200 times greater than the critical Rayleigh number required for the onset of convection, but probably still ~10 times smaller than the average value for the Earth's mantle. We use a timestep of 10^6 years, one quarter of the Courant condition, and start from small random initial perturbations in the entropy (temperature) field[7].

The resulting time-averaged heat flows through the inner and outer boundaries are 1×10^{13} W and 5×10^{13} W, respectively. Thus, ~80% of the heat conducted through the outer boundary is generated in the model mantle. The total heat flow through the outer boundary is comparable to the heat flow through the Earth's surface. In the bulk of the model mantle >90% of the heat flow is convective and <10% is conductive. Maximum convective velocities in the model are ~30 mm yr^{-1}, comparable to the observed velocities of tectonic plates.

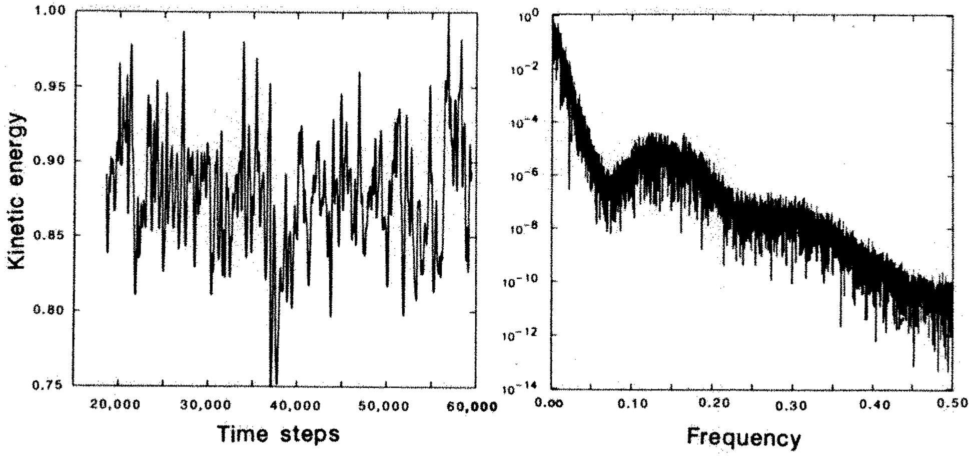

FIG. 1 Total kinetic energy as a function of the timestep and its power spectrum as a function of frequency (in cycles per second scaled by the timestep). Both plots are normalized to unity. For this long-time integration we truncated the spherical harmonic expansions at $l=63$ instead of 85.

The total kinetic energy in the model mantle is plotted in Fig. 1 as a function of time for the last 40,000 timesteps of the calculation. The large amplitude variation illustrates the time dependence of the solution; however, the basic qualitative features of the convection do not change over this period of time. Also plotted in Fig. 1 is the square of the Fourier transform of the kinetic energy time series as a function of frequency. The broadband distribution of power is indicative of a chaotic system. In addition, the phase space representation of kinetic energy as a function of its time rate of change (not displayed here) shows no limit cycle or torus, indicating chaos. Two popular methods for calculating the fractal dimension[14,15] failed to converge, suggesting a high fractal dimension for our three-dimensional convection. Our results support the conclusion, made on the basis of two-dimensional calculations[16-18], that mantle convection in the Earth is chaotic. Our results suggest, however, that mantle convection is much more chaotic than the low-fractal-dimension solutions obtained with the two-dimensional models using low Rayleigh numbers and no internal or viscous heating.

A typical snapshot of the spatial patterns of the temperature and velocity fields on three different surfaces of constant radius is shown in Fig. 2. In the upper part of the sphere, cold dense fluid tends to converge and sink in long narrow sheets surrounded by a weak background of warm buoyant diverging upflow. This thin sheet-like downflow, initiated by the thin thermal boundary layer at the top, tends to break up with increasing depth into cylindical downflow plumes that squeeze the hot fluid between them as they impinge on the inner boundary forcing hot cylindrical upflow plumes at the vertices of the connected network of warm fluid. These upflow plumes expand in the upper part of the sphere forming the weak background of upwelling there.

The formation of the upwelling plumes at the intersections of warm linear thermals is similar to the spoke pattern of convection observed in laboratory and numerical studies of plane-layer convection with constant viscosity and all heating from the bottom of the fluid layer[2,4,19]. Purely bottom-heated convection in spherical shells does not result in such spoke patterns and in fact maintains upwelling plumes surrounded by downwelling sheets over the entire depth of the shell; the contrast with plane-layer convection is primarily because of the breaking of the mid-plane symmetry by the spherical geometry[7,9,20]. Thus, the similarity of our internally heated solutions to bottom-heated plane-layer convection implies that the mid-plane symmetry is partially restored by internal heating in the spherical shell (by equalizing the temperature drops across the top and bottom boundary layers, for example). Unlike plane-layer convection, however, the network of linear thermals is more prominent in the temperature field (which is what the shadowgraphs in laboratory experiments detect), whereas the strong upwelling motion is primarily confined to cylindrical plumes.

Three snapshots, 200 million years apart, of the spatial patterns of the temperature and velocity fields in a typical cross-

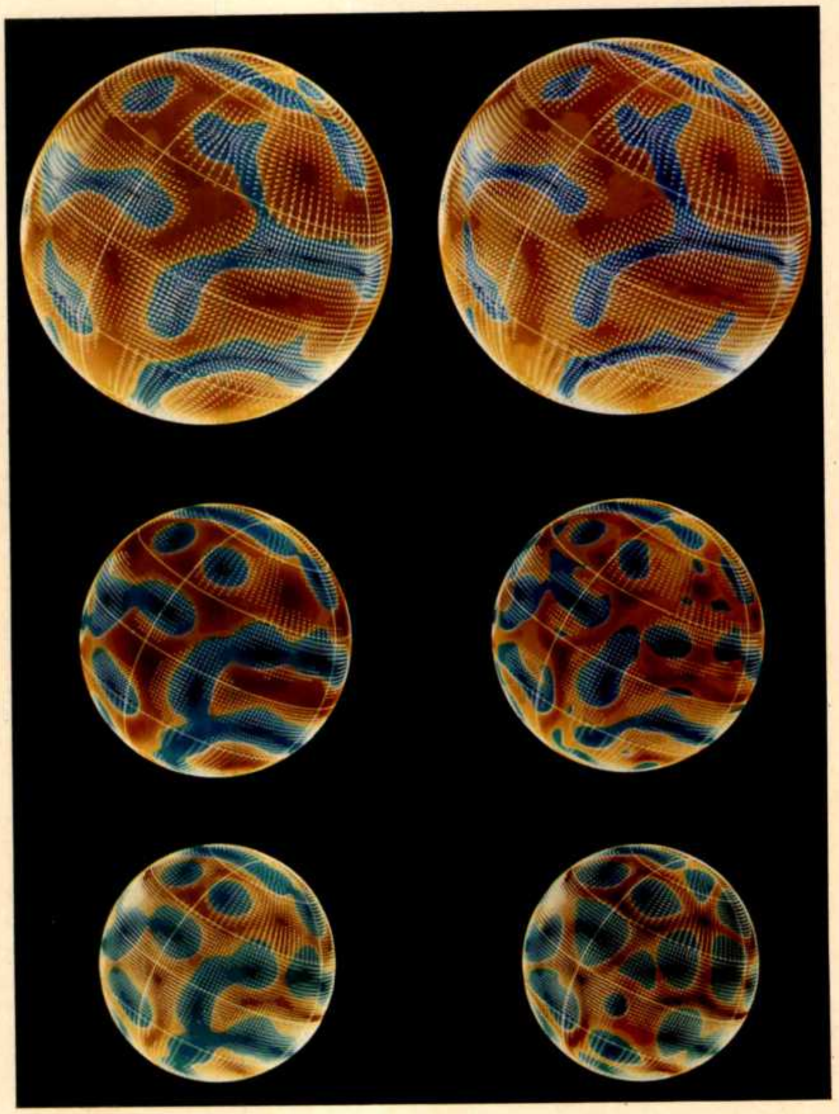

FIG. 2 Plots (all at the same timestep) of convective velocities and temperatures in three different constant-radius surfaces (5,940, 4,350 and 3,770 km). These spherical surfaces are scaled according to their radii. The colours in the three plots on the left represent the radial component of velocity with a contour increment of 1.5 mm yr^{-1}. Reds and yellows represent upflow (a maximum of 12.0 mm yr^{-1}); blues represent downflow (a maximum of 19.5 mm yr^{-1}). The colours in the three plots on the right represent the temperature relative to the spherically averaged value at each radius with a contour increment of 50 K. Reds and yellows represent hot fluid (a maximum of +400 K); blues represent cold fluid (a minimum of −650 K). The arrows represent the direction and amplitude of the horizontal velocity in these surfaces. All are scaled in the same way, with a maximum of 20 mm yr^{-1}. Velocities less than 2 mm yr^{-1} are not plotted.

sectional slice through the spherical shell are illustrated in Fig. 3. A typical cold boundary layer instability at '12 o'clock' is developing in the first (top) plot. It has sunk to mid-depth in the second (middle) plot, and is spreading along the lower boundary in the third (bottom) plot. Figures 2 and 3 show how the spatial scale of the temperature is smaller than that of the velocity (owing to the small thermal diffusivity compared to kinematic viscosity) and how at certain times and places relatively hot fluid is dragged downward.

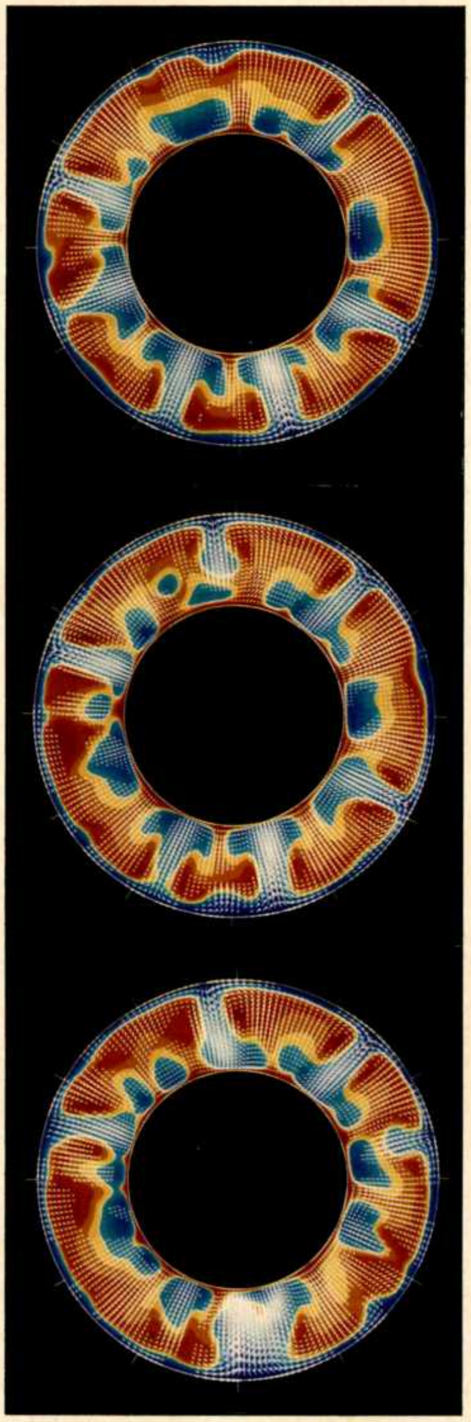

FIG. 3 Plots (in the same cross-sectional slice through the three-dimensional shell) of convective velocities and temperatures at three different times separated by 200 timesteps (200 million years) beginning with the top plot. The colours represent the temperature relative to the radially dependent adiabatic temperature profile with a contour increment of 50 K. Reds and yellows represent hot fluid (a maximum of 300 K); blues represent cold fluid (a minimum of −750 K). The arrows represent the velocities in this cross-sectional surface and are scaled the same way as those in Fig. 2.

A movie of this numerical simulation shows the convection pattern continuously changing, with the hot upflow passively reacting to the dominant cold downflow. The long downflow sheets in the upper part of the sphere sometimes contract or break up, forming cylindrical-like downwelling features and at other times the downflow sheets link up forming even longer sheet-like features. Downwellings form spontaneously over regions that are warm at depth. Although the convection pattern is very chaotic, the basic spatial and temporal characteristics of the convection do not change after the decay of the initial transients.

We can compare these results to our previous calculations with 50% and 80% internal heating[9,10] which were run at lower Rayleigh numbers and at a resolution a factor of two lower in both latitude and longitude. Although the previous patterns were time-dependent, the present results are more temporally complex and definitely chaotic. In our earlier calculations convection patterns tended to be anchored to the upwelling plumes which were relatively steady in number and location. In contrast, the upwelling plumes in our present calculations are more variable in number and location owing to their interaction with chaotic downwelling. At even higher Rayleigh numbers than in the present calculation (stronger thermal driving relative to viscous and thermal diffusion), convection will probably have greater spatial and temporal structure and modelling will require still better numerical resolution. Higher numerical resolution (and therefore more computing resources) will also be required when three-dimensional models account for variable viscosity, tectonic plates and phase and chemical transitions. It is difficult to predict how these needed improvements will alter the structure of the simulated convection. It is, however, encouraging that the sheet-like downwellings seen in our previous calculations at 80% internal heating[10] also dominate the structure of convection here. This qualitative agreement gives us confidence that this downwelling pattern will continue to be an important feature as models of mantle convection become more realistic.

If the numerical model presented here is sufficiently representative of the Earth's mantle, the numerical results have significant implications for the nature of mantle convection. Convection in the model is predominantly controlled by the largely sheet-like downwellings, suggesting that the descending slabs in the Earth are the main drive for plate motions and mantle convection[21]. The prominent role of downwellings in convecting systems heated strongly from within has also been demonstrated in other numerical studies[2,4,8-10] and in laboratory experiments[22,23]. The lengthening of downwellings in the model along their axes suggests the possibility that subduction zones on Earth may evolve by propagation along their strike[4]. In our model, the downwelling sheets also drift perpendicular to their axes, similar to the lateral migration (perpendicular to strike) of descending slabs through the mantle[24]. Because subducting slabs probably drive plate tectonics, their chaotic migration and propagation (inferred from these numerical calculations) may also have a role in the apparently random dispersal and aggregation of continents.

As the upflow in our calculations takes the form of cylindrical plumes, our results support the evidence that mid-ocean ridges are the result of passive rifting[9,25]. The upwelling plumes in our model broaden with height. A temperature-dependent viscosity would probably focus the thermal and velocity anomalies of plumes into smaller-diameter cylindrical structures and flatten the heads of plumes more dramatically as they encounter the base of the strong lithosphere[26,27]. These plumes probably weaken the lithosphere and determine where the lithosphere tears and spreads apart in response to slab pull. This may be one reason why many hot spots are preferentially located near mid-ocean ridges[28]. □

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Enhancement of nitrogen deposition to forest trees exposed to SO_2

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DEPOSITION of atmospheric nitrogen compounds, particularly ammonia, is postulated to have a variety of deleterious effects[1], including damage to soils, trees and other vegetation[1–6]. We report here that fumigation of coniferous trees in the open air with sulphur dioxide (13 and 22 p.p.b.–parts in 10^9 by volume) produced an increased deposition of atmospheric NH_3 to foliage. A mechanism for co-deposition of NH_3 and SO_2 to wet surfaces has been suggested[7,8]. As concentrations of NH_3 at the site are not unusually high (annual mean, 4 p.p.b.)[9], this mechanism may enhance nitrogen deposition over large areas of Europe. It may also account for some results of experimental fumigations previously attributed to SO_2 alone.

The experimental site is located at Liphook, Hampshire, UK and uses a technique previously developed to fumigate cereals with SO_2 (ref. 10). In 1985 seven plots (Fig. 1) were planted with seedlings of Scots pine (*Pinus sylvestris*), Sitka spruce (*Picea sitchensis*) and Norway spruce (*Picea abies*)[11]. Concentrations for SO_2 treatments were multiples (1.5 and 3 times) of sequential hourly mean concentrations measured in 1979–1980 at a rural site in central England[12]. Target values for ozone treatments were a constant multiple (1.5 times) of the measured ambient O_3 at the site. Including ambient concentrations, this gives a 2×3 factorial design with one additional ambient plot (Fig. 1). Exposure to SO_2 commenced in May 1987 and O_3 fumigation occurred from March–December 1988 and May–December 1989.

Each October, eight trees of each species per plot were harvested for biometric and chemical analysis. The foliar content of nitrogen, sulphur and cations was determined by standard methods[13]. In summer 1989, throughfall collectors (diameter, 0.9 m) were fitted to four trees each of Scots pine and Sitka spruce in plots 2–7. Samples were collected fortnightly and analysed separately.

In October 1988 the foliar nitrogen content of Norway spruce showed a clear trend with SO_2 treatment, being 1.7 times higher in the high-SO_2 plots than in the ambient plots (Fig. 2). Sulphur content of the needles also reflected the SO_2 treatments (Fig. 2). A similar trend in foliar nitrogen was observed in Sitka spruce, but not in Scots pine. Needles of Norway spruce on plots without SO_2 fumigation were below the deficiency level of 12 mg N g^{-1} in October 1988 (Fig. 2); needles of Sitka spruce on all plots were likewise deficient, whereas those of Scots pine remained above the deficiency level of 11 mg N g^{-1} (ref. 14). Estimates of the total nitrogen content of needle biomass in October 1988 revealed that the Norway spruce and Sitka spruce contained more nitrogen (7.8 kg ha^{-1} and 12.8 kg ha^{-1}, respectively) in the 22 p.p.b. SO_2 plots than trees in ambient plots.

There are several mechanisms that might explain this difference in nitrogen, including N_2 fixation, litter decomposition, reallocation from roots, changes in understorey vegetation and atmospheric inputs. Studies of the soil nitrogen cycle[15] found no evidence for differences in N_2 fixation or nitrogen mineralization. It is possible that SO_2 effects have suppressed the nitrogen demand of understorey vegetation, but there is no evidence for this. Likewise, studies of below-ground biomass have shown no indication of treatment effects (although with large errors), and reallocation of nitrogen from needles prematurely lost in SO_2-treated plots would not supply enough nitrogen to explain the effect. The most probable explanation for the increased nitrogen appears to be a known mechanism for SO_2 enhancement of NH_3 deposition to wet surfaces[7,8]. The solubility of SO_2 or NH_3 alone is limited by the pH of the acidic or basic solution they form, respectively, whereas with both gases together, the pH can remain at non-limiting values, so enhancing solubility and deposition. Evidence to support this comes from measurements of ammonium ion in throughfall and measured deposition to water surfaces within the plots.

Ammonium in throughfall has been measured from July 1989

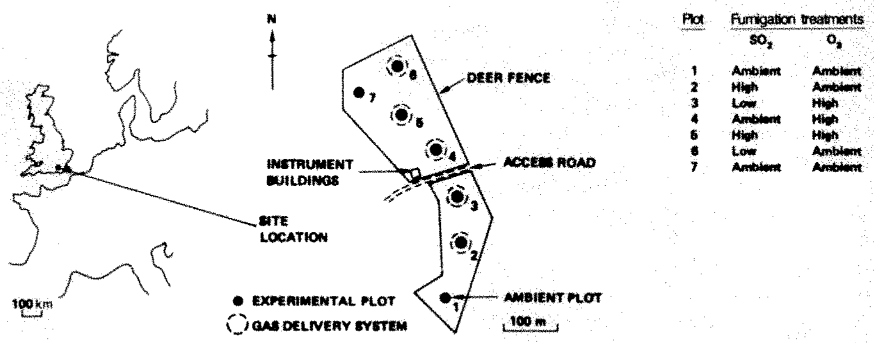

FIG. 1 Map of Europe showing the location of the study site, the layout of the experimental plots and gaseous treatments. The plots were planted with 196 seedlings of each species at 1-m spacing in a 25-m diameter area within a 50-m diameter circle of gas sources[11].

TABLE 1 Chemical composition of rain and throughfall (including stemflow) below Scots pine and Sitka spruce in open-air fumigation plots from 12 July 1989 to 24 January 1990

		Treatment concentration (p.p.b.)		Throughfall concentration (μeq l^{-1})				
Species	Plot	SO_2	O_3	NH_4^+	NO_3^-	SO_4^{2-}	H^+	pH
Pinus sylvestris	2	21	19	192 (60)	115 (24)	1,016 (146)	276 (30)	3.6
	3	13	24	118 (25)	150 (39)	693 (86)	174 (24)	3.8
	4	6	24	93 (16)	169 (41)	535 (82)	100 (19)	4.0
	5	21	24	185 (37)	215 (39)	1,294 (136)	333 (38)	3.5
	6	12	19	86 (14)	119 (25)	720 (87)	185 (19)	3.7
	7	5	19	101 (23)	146 (35)	353 (47)	73 (15)	4.1
Picea sitchensis	2	21	19	102 (18)	173 (31)	1,015 (125)	289 (34)	3.5
	3	13	24	73 (11)	99 (20)	704 (78)	174 (23)	3.8
	4	6	24	93 (14)	97 (22)	445 (61)	39 (9)	4.4
	5	21	24	139 (23)	149 (26)	1,056 (97)	282 (32)	3.6
	6	12	19	95 (19)	107 (21)	784 (88)	172 (23)	3.8
	7	5	19	69 (14)	110 (24)	447 (92)	50 (10)	4.3
Rainfall, north site		5	19	51 (10)	33 (9)	90 (10)	19 (7)	4.7
Rainfall, south site		5	19	49 (15)	71 (22)	114 (17)	31 (18)	4.5

NH_4^+ was determined by the indophenol blue colorimetric method[13], and NO_3^- and SO_4^{2-} by ion chromatography. Values are the means from four replicate throughfall collectors sampled on 10 occasions (at fortnightly intervals unless there was no rainfall), with standard errors in brackets; pH values are calculated from the mean H^+ concentration. Gaseous concentrations are the mean values over the period of throughfall sampling. Mean concentrations of ambient NO and NO_2 were 5 and 8 p.p.b. respectively. Ozonized air was water-scrubbed to remove contaminant N_2O_5 (ref. 11) during this period and the preceding fumigation in 1989.

TABLE 2 Chemical composition of water samples exposed in open-air fumigation plots during March 1990

		Treatment concentration (p.p.b.)		Concentration (μeq l^{-1})				
Period	Plot	SO_2	O_3	NH_4^+	NO_3^-	SO_4^{2-}	H^+	pH
2–7 March (125 h)	1	3	25	53.8 (5.7)	3.9 (0.3)	20.1 (0.5)	0.5 (0.1)	6.3
	2	35	26	76.6 (5.7)	5.3 (0.3)	101.7 (2.5)	128.1 (0.8)	3.9
	5	38	34	78.6 (5.6)	5.4 (0.2)	109.8 (4.3)	145.7 (6.9)	3.8
	7	3	26	53.3 (0.8)	4.3 (0.2)	19.2 (0.5)	0.5 (0.1)	6.3
7–13 March (139 h)	1	3	21	83.7 (4.9)	14.5 (0.4)	48.7 (2.3)	16.7 (0.7)	4.8
	2	33	22	113.9 (5.2)	18.0 (0.3)	112.1 (6.7)	97.0 (8.3)	4.0
	5	35	32	112.7 (2.1)	17.5 (0.9)	144.1 (6.7)	151.3 (13.4)	3.8
	7	3	22	82.7 (4.0)	13.4 (0.7)	61.5 (1.1)	36.8 (2.7)	4.4

Deionized water (25 ml) was exposed in open polystyrene Petri dishes (8.7 cm diameter) at 1.5 m height and protected from rainfall in the ambient and high-SO_2 plots. Water levels were maintained daily. NH_4^+, NO_3^- and SO_4^{2-} concentrations are mean values (with standard errors) for four replicate dishes, two each on the north and south edges of the trees, 12.5 m from the gas outlets (Fig. 1), compared with water in two sealed control dishes. Analytical methods are given in Table 1; pH values are calculated from the mean H^+ concentration. Dilute sulphuric acid (25 ml; 0.17 mg l^{-1} SO_4^{2-}) kept in closed Petri dishes for 6 days showed no increase in NH_4^+ concentration. NH_4^+ analysis by the indophenol blue colorimetric method[13] may also detect volatile amines[22] and results were confirmed using ion chromatography. Mean concentration of ambient NO was <2 p.p.b. during each period and NO_2 was 3 p.p.b. from 3–7 March and 5 p.p.b. from 7–13 March. There is no evidence for NO_3^- deposition from N_2O_5 contamination of the ozonized air[11], which was water-scrubbed before fumigation during these periods.

and shows concentrations in high-SO_2 plots 1.9-times higher for Scots pine and 1.7-times higher for Sitka spruce than in the ambient plot (Table 1). Some of this NH_4^+ may have been leached from internal sources, but it seems likely that a large fraction results from wash-off of dry deposited NH_3 following co-deposition with SO_2 (ref. 6). These data therefore suggest that dry deposition of NH_3 is enhanced in SO_2 fumigated plots. The NH_4^+ in throughfall represents the excess of deposition and leaching over uptake, and this excess may have been insufficient to enhance the throughfall concentrations in the low-SO_2 treatment plots.

The deposition of NH_3 was evaluated by exposing dishes of deionized water for several days in the ambient and high-SO_2 plots. Analysis for NH_4^+ (Table 2) suggests an additional deposition of 27 and 31 ng N cm^{-2} d^{-1} to the water surfaces in the high-SO_2 plots compared with those in the ambient SO_2 plots during the two periods. The much smaller increase in NO_3^- may result from reactions between SO_2 and NO_2 in solution to enhance NO_2 deposition[16]. These data imply that SO_2 may enhance NH_3 deposition to all ecosystem surfaces, including leaf surfaces and mesophyll cells, bark and soils[17-19]. Calculated nitrogen deposition rates of ~10–20 kg ha^{-1} yr^{-1} are obtained using a deposition velocity of NH_3 to vegetation of 5–10 cm s^{-1} (ref. 20), assuming a concentration of 1 p.p.b. NH_3 (the lower end of the range of annual mean concentrations reported for the UK[9]). Although throughfall composition (Table 1) and deposition to water surfaces (Table 2) strongly suggest that NH_3 is the source of the additional foliar nitrogen, some NH_3 may originate from the vegetation[21], and then be captured by the foliage[22].

Enhanced nitrogen deposition of this magnitude has important implications because 10–20 kg ha^{-1} N has been proposed as a 'critical load' that should not be exceeded if damage to forest ecosystems is to be avoided[23]. The potential for enhanced

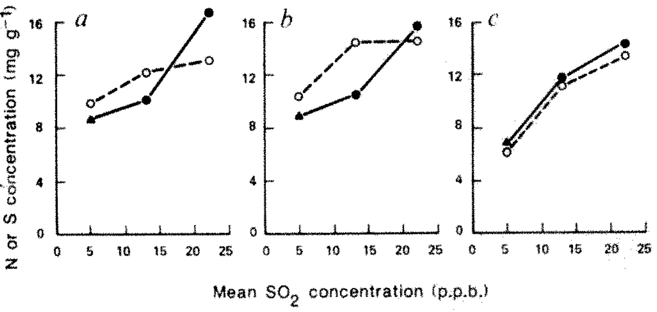

FIG. 2 Foliar concentrations of nitrogen and sulphur in Norway spruce trees exposed to SO$_2$ and O$_3$ by open-air fumigation and sampled in October 1988 (*a*, N in 1987 needles; *b*, N in 1988 needles; *c*, S in 1988 needles). Values are means of 8 trees for the 5 fumigated plots (○, ●), and 16 trees for the 2 unfumigated ambient plots (▲). Open symbols represent plots receiving ozone fumigation (30 p.p.b.) and filled symbols indicate ambient O$_3$ plots (24 p.p.b.). Exposure concentrations are annual means for October 1987 to September 1988. Ozone was generated from electric discharge in dry air forming contaminant N$_2$O$_5$ (ref. 11) that was removed (in 1989 only) by recirculating water in a packed-bed scrubber. Nitrogen content of O$_3$-fumigated needles may be affected by N$_2$O$_5$ deposition before 1989, and by O$_3$ oxidation of SO$_3^{2-}$ which may enhance NH$_3$ co-deposition with SO$_2$ (refs 7, 8) at pH values of surface moisture above 4.7. Nitrogen content was assessed after digestion with H$_2$O$_2$-H$_2$SO$_4$ using the indophenol blue colorimetric determination of NH$_4^+$ (ref. 13). Sulphur content was determined after ashing with HNO$_3$ at 550 °C, using inductively coupled plasma emission spectrometry.

NH$_3$ deposition may be greater for mature forest trees, for which the mean leaf area index (LAI) is 3-5 (ref. 24), as compared with the trees in this experiment, for which the LAI is 1-2.5. Some results of experimental SO$_2$ fumigation or ambient air filtration may need to be reassessed, depending on whether charcoal filters were used, which remove half of the NH$_3$ (ref. 25), or materials containing KMnO$_4$, which may remove it all. In addition, the use of nitrogen-sufficient plants may obscure the effect of increased atmospheric nitrogen supply on foliar nitrogen concentration[26]. The nitrogen-sufficient Scots pine in this study did not show the same response as the spruces. Enhanced NH$_3$ deposition could also explain the increased foliar nitrogen observed during open-air SO$_2$ fumigation of cereals[10,27], increased performance of insects[27], mildew[28], and increased activity of glutamate dehydrogenase[29], all previously reported as solely SO$_2$ effects. The importance of NH$_3$ and NH$_4^+$ deposition in areas of high-NH$_3$ emissions is well known[1-6], but these data indicate that it may be important over wide areas where relatively low concentrations of SO$_2$ and NH$_3$ co-exist. □

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Acceleration of senescence in the collared flycatcher *Ficedula albicollis* by reproductive costs

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SENESCENCE is the innate deterioration of an organism that leads to a decline in its fitness components such as fecundity and survival expectations with advancing age[1,2]. Williams[3] proposed that the evolution of senescence could be understood in terms of age-specific pleiotropic gene action, where a mutation increases fitness early in life at the expense of reducing it later on. The basic idea, first proposed by Medawar[1], is that mutations acting early in life are more sensitive to selection than those acting later, as the early effects would be expressed in more individuals than would the late effects. A cost of reproduction, where an increase in reproductive rate reduces future survival or fecundity, could be the kind of pleiotropic gene action that generates senescence[2,3]. Evidence exists for such costs in animals and plants[4-8], but their role in the evolution of senescence is so far not clear[2]. Most empirical work has been with fruit-flies in laboratory studies, where selection experiments indicate negative genetic correlations between early and late fitness components[9-12]. But it is not certain how applicable the results of such studies are to natural populations. Here we report that the clutch size of older individuals in the collared flycatcher *Ficedula albicollis* is affected by their reproductive effort early in life. This provides empirical support for Williams's idea that costly reproduction accelerates senescence for fertility in a vertebrate species under natural conditions.

Time, energy and nutrients are often limited for individuals and have to be allocated between somatic maintenance, growth and reproduction. General life-history theory[4,13,14] predicts that a high reproductive effort early on in life should be costly for an individual and lead to reduced lifespan or subsequent fertility. There have been two approaches to the investigation of these costs: genetic studies[3,4,15], and phenotypic studies using environmental manipulations of reproductive rate[4,16-17]. Here we report the use of the second approach.

We have studied the population biology of the collared flycatcher on the southern part of the island of Gotland[18,19] in the Baltic from 1980 to the present. The collared flycatcher *Ficedula albicollis* on the island of Gotland forms an isolated population so that survival and reproduction of both parents and their offspring can be assessed throughout their entire lifespan[18,20].

The reproductive performance of collared flycatcher females increased during early life and decreased at older ages, although there was no significant change in survival probability (Fig. 1). A similar pattern has also been observed for many other bird species[21]. Because we have found no evidence of a higher frequency of external injuries or defects in these old birds, the

TABLE 1 Effects of age of first breeding and current age on clutch size in female collared flycatchers

	Cross-section		Longitudinal	
n	516		165	
Source	*F*-value	*P*	*F*-value	*P*
Age (current)	1.27	0.282	1.93	0.149
Age (first breeding)	6.19	0.013	8.96	0.003
Interaction	0.88	0.414	0.11	0.894

Two-way factorial ANOVA; mean values, standard errors and sample sizes are shown in Fig. 2. We regarded individuals that were not captured by us during their first year of life as nonbreeders. This category probably included individuals that actually bred during their first year, but because breeding dispersal is very low[19], only very few of our putative nonbreeders are likely to have bred outside the study area. Although this category might include a few birds that did have some reproductive effort in their first year, our comparison for differences is conservative.

TABLE 2 Effects of age and brood size manipulations on clutch size in female collared flycatchers in the three years after the experiment

	All birds				Only one-year-old			
	Cross-section		Longitudinal		Cross-section		Longitudinal	
n	201		74		112		50	
Source:	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Age	0.85	0.43	1.84	0.166	0.19	0.83	0.31	0.73
Treatment	8.15	0.005	19.1	0.0005	4.27	0.041	4.87	0.002
Interaction	2.97	0.054	0.23	0.79	1.76	0.18	0.16	0.85

Two-way factorial ANOVA analyses for all birds irrespective of age (age did not differ between groups (U-test, $P > 0.5$) and for birds that were one year old during the manipulation are presented. Mean values, standard errors and sample sizes are shown in Fig. 2*b, c*. We used individuals from a previously published brood size experiment[6] that had either increased or reduced broods (one or two young). Because these manipulations were found to affect both subsequent clutch size[6] and fledgling success (L.G., unpublished results), the number of individuals of older ages that could be used was reduced because many were manipulated in several years. Because the number of individuals is small in the older age classes we could only use individuals up to the age of 4 years (that is, 3 years after the experiment). A one-way ANOVA showed a significant difference between enlarged broods and both control and reduced broods ($P < 0.05$, Scheffe's test) in years after the experiment, whereas there were no significant difference between reduced and control broods. These results are probably because reduced broods have heavier young[22] with higher survival[6], thereby reducing any difference in effort between reduced and control females. We therefore pooled the data of control and reduced broods.

decrease in reproductive performance is probably caused by some innate deterioration of the individual. Thus, collared flycatcher females older than 3 years clearly show signs of senescence for fertility.

By what processes could a seemingly maladaptive character like senescence for fertility evolve? A cost of reproduction could be a candidate for the gene action proposed by Williams[2,3]. To test Williams's idea that reproductive effort early in life affects fecundity late in life we used two methods. First, we compared birds breeding for the first time at the age of 1 year to those that started to breed at 2 years of age. Second, we compared individuals with experimentally enlarged brood size to those with reduced or unmanipulated brood size. The first approach is purely correlative, and therefore less powerful than the second[4-6].

Collared flycatcher females that did not breed during their first year laid larger clutches throughout their subsequent life than did females starting to breed in their first year (Table 1; Fig. 2*a*). It is not likely that 'first-' and 'second-year' breeders represent two different strategies (that is, genetic lines with late breeding and high fecundity versus early breeding and low fecundity) as the number of breeding seasons is the most important factor affecting lifetime reproductive success (LRS) in the collared flycatcher[18], thereby promoting a strong selection for breeding in the first year of life[18]. Furthermore, a comparison of birds used in this analysis showed that first-year breeders had higher LRS (1.24 ± 0.08 recruits) than second-year breeders (0.90 ± 0.14 recruits; $z = -2.03$, $P < 0.05$, Mann-Whitney U-test). The most likely explanation therefore is a cost of early reproduction to be paid as reduced reproductive performance later in life. This explanation is strongly supported by the brood size manipulation where females with enlarged brood size laid smaller clutches later in life than those with a reduced or unmanipulated brood (Table 2, Fig. 2*b, c*).

One basic premise in Williams's ideas on the evolution of senescence is that there should be selection for high early fertility. This was true for age of first breeding (see above). Furthermore there is also selection for a large clutch size among one-year-old females (regression; LRS = 0.19 × clutch size − 0.35, $F = 7.47$, $n = 349$, $P < 0.01$).

Our results demonstrate that there is a permanent effect of early reproductive effort, and that an increase in the rate of senescence for fertility is therefore induced. The mechanism of the effect is at present not clear; it is not due to risky reproduction through ecological risks, a mechanism defined by Partridge[2], because that should affect only survival. It is possible to imagine a mechanism of physiological exhaustion lasting from year to year such as, for example, delayed moult, migration and start

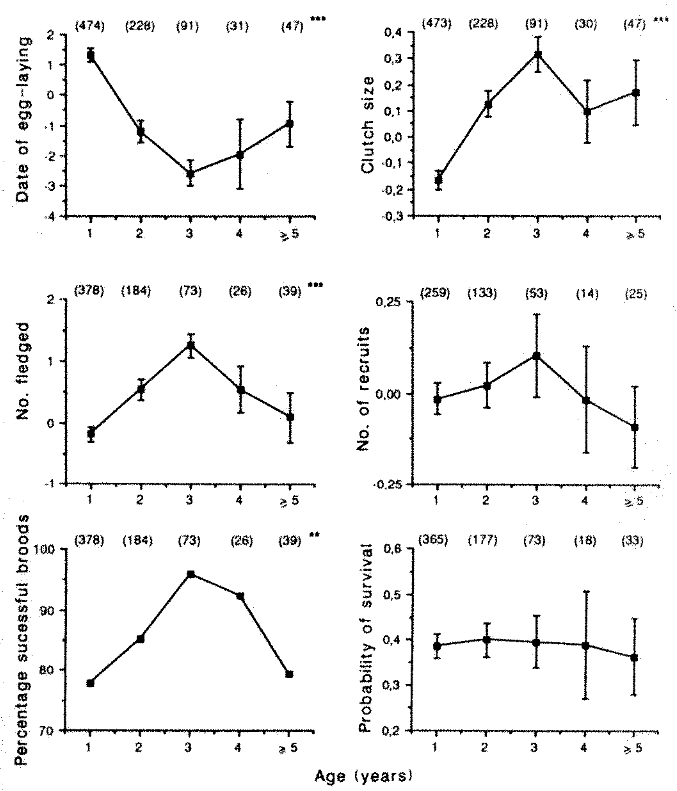

FIG. 1 Cross-sectional data of age and reproductive performance in collared flycatcher females breeding between 1980 and 1987. All data, except percentage of successful broods and probability of survival were standardized (mean = 0) for each year. Sample sizes are shown in brackets and *P* values are indicated as ** < 0.01, *** < 0.001. The test used was one-way ANOVA, except for percentage successful broods and survival rates, where a G-test was used. In the age-class of ≥5-year-old females we included birds with an unknown exact age at first trapping and therefore the sample size was larger than for 4-year-old females. The shown parameters are added effects of age, but, also when creating independent parameters by correcting for date of egg laying (residual analysis), clutch size and number of fledged young per laid egg showed the same relationship to age ($P < 0.01$), but number of recruits per fledged young was not significantly related to age.

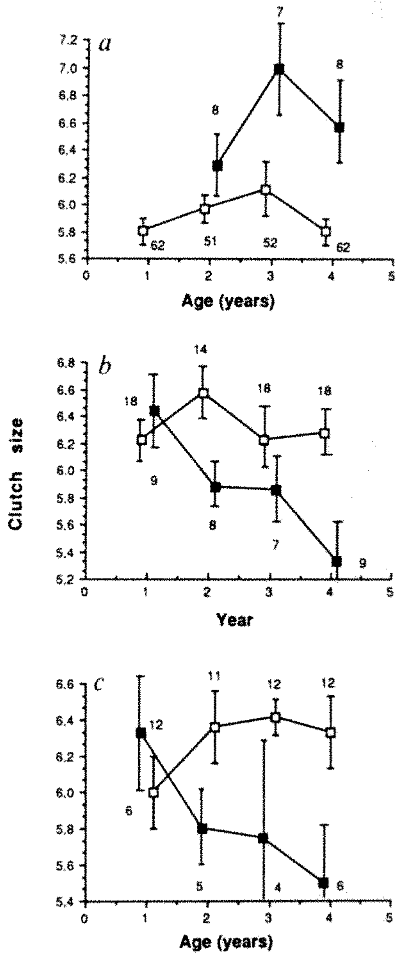

FIG. 2 Clutch size of collared flycatchers of different age *a*, using longitudinal data for females starting to breed as one (open) versus 2-year-olds (filled), *b*, females with enlarged (open) versus control broods (filled) irrespective of age at manipulation and *c*, females with enlarged (open) versus control broods (filled) manipulated as 1-year-olds. All data are ±1 s.e.m. and numbers are sample sizes. Manipulations were done at year one in *b* and age 1 year in *c*. The earlier decline of clutch size for control individuals in *b* compared with *c* arises because many birds were here older than 1 year when manipulated. In fact age for this sample is ~4 years at three in *b*. This coincides with the start of senescence for fertility shown in Fig. 1 and in *a* and *c*. The displayed pattern cannot be due to between-year differences in clutch size as both groups were equally represented in all years. Also the changes in clutch size could not be explained by a change in date of egg laying because we could not find any differences in start of breeding later in life for our compared groups. The only significant differences were in the year following the manipulation, as birds from the enlarged group started, on average, to breed later than control birds (mean date in May 27.0 resp. 24.7 days, $t=2.21$, degrees of freedom $=110$, $P<0.05$). This difference, however, did not persist in the following years when clutch size differed even more between groups.

of breeding with lowered territory quality and breeding performance as a consequence. However, it is not obvious how that effect can linger on to later years.

A pleiotropic gene action, as proposed by Williams[3], would involve an initial genetic effect, for example, on reproductive rate early in life, and a subsequent decline in reproductive probabilities achieved as lower fertility and/or survival. Our manipulation and the birds' decision about when to start breeding have mimicked the early genetic effect and the subsequent drop in clutch size that we observe would then presumably be caused by ordinary physiological effects of the early effort. Our results give support for Williams[3] evolutionary explanations of senescence in that we demonstrate that when there is a strong selection for an early high fertility in a natural population there is also a link between early effort and late performance. □

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Clustering of L-type Ca^{2+} channels at the base of major dendrites in hippocampal pyramidal neurons

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INTEGRATION and processing of electrical signals in individual neurons depend critically on the spatial distribution of ion channels on the cell surface. In hippocampal pyramidal neurons, voltage-sensitive calcium channels have important roles in the control of Ca^{2+}-dependent cellular processes such as action potential generation[1,2], neurotransmitter release[3], and epileptogenesis[4,5]. Long-term potentiation of synaptic transmission in the hippocampal pyramidal cell, a form of neuronal plasticity that is thought to represent a cellular correlate of learning and memory[6,7], is dependent on Ca^{2+} entry mediated by synaptic activation of glutamate receptors that have a high affinity for NMDA (*N*-methyl(-D-aspartate) and are located in distal dendrites[8,9]. Stimuli causing long-term potentiation at these distal synapses also cause a large local increase in cytosolic Ca^{2+} in the proximal regions of dendrites[10]. This increase has been proposed to result from activation of voltage-gated Ca^{2+} channels[10]. At least four types of voltage-gated Ca^{2+} channels, designated N, L, T and P (refs 11, 12), may be involved in these processes. Here we show that L-type Ca^{2+} channels, visualized using a monoclonal antibody, are located in the cell bodies and proximal dendrites of hippocampal pyramidal cells and are clustered in high density at the base of major dendrites. We suggest that these high densities of L-type Ca^{2+} channels may serve to mediate Ca^{2+} entry into the pyramidal cell body and proximal dendrites in response to summed excitatory inputs to the distal dendrites and to initiate intracellular regulatory events in the cell body in response to the same synaptic inputs that cause long-term potentiation at distal dendritic synapses.

The different classes of voltage-gated Ca^{2+} channels in neurons have distinct physiological and pharmacological properties[13]. N-type Ca^{2+} channels are activated at relatively

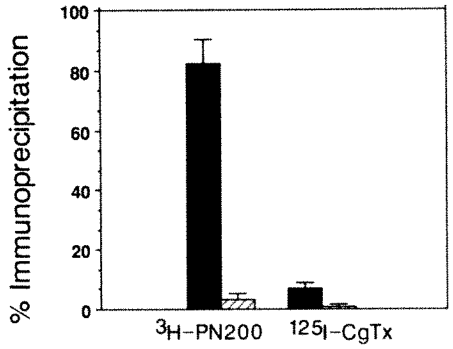

FIG. 1 Immunoprecipitation of L- and N-type voltage-dependent Ca^{2+} channels by MANC-1 (■) and nonimmune IgG (▨). Specific binding sites for [^{3}H]PN200-110 (a dihydropyridine) and ^{125}I-labelled CgTx, both prepared from rabbit hippocampal synaptic plasma membranes, immunoprecipitated to define L- and N-type channels, respectively.

METHODS. Hippocampal synaptic plasma membranes and L-type Ca^{2+} channels were prepared as previously described[26]. For L-type channels, membranes were prelabelled with 2 nM [^{3}H]PN200-110 in the absence (total binding) and presence (nonspecific binding) of 1 μM unlabelled PN200-110. The membranes were solubilized (2 mg ml^{-1} in 1% digitonin) and the extracts subjected to wheat-germ agglutinin-Sepharose chromatography. Peak fractions, containing the total and corresponding nonspecific binding, were saved and used for immunoprecipitation experiments. For N-type channels, hippocampal membranes were solubilized as above, diluted to a protein concentration of 0.2 mg ml^{-1} in 50 mM HEPES–Tris buffer, pH 7.4 (ref. 27) and divided into two equal samples. Vehicle or unlabelled CgTx (1 μM final) were added to define total and nonspecific binding, respectively, and allowed to incubate for 10 min at 22 °C. ^{125}I-labelled CgTx (prepared as in ref. 28) was then added to both samples and allowed to incubate for 10 min at 22 °C. Aliquots from these postlabelled samples were then used in immunoprecipitation experiments. Immunoprecipitation of either [^{3}H]PN200-110 or ^{125}I-labelled CgTx binding sites was performed as previously described[26]. Each reaction contained 1 μM MANC-1 (purified from ascites using Protein-A Sepharose) or non-immune mouse IgG, 0.5–2.5 fmol [^{3}H]PN200-110 binding sites or 3,500 to 14,000 c.p.m. (1.0–4.3 fmol) of specifically bound ^{125}I-labelled CgTx, 0.1% digitonin, 75 mM NaCl, 25 mM HEPES–Tris buffer, pH 7.4, 50 mM NaH_2PO_4, 20 mM NaF, 2.5 mM NaEDTA, and protease inhibitors (aprotinin, 2 μg ml^{-1}; O-phenanthrolin, 1 mg ml^{-1}; pepstatin A 1 μg ml^{-1}; benzamidine, 1 mg ml^{-1} and leupeptin, 1 μg ml^{-1}). This mixture was incubated at 4 °C for 4 h and 100 μl of 10% Protein-A Sepharose-which had been pre-equilibrated with rabbit versus mouse IgG and BSA, and washed with the above buffer—were added for 1–2 h at 4 °C. The mixture was centrifuged and the supernatant collected. The pellet was washed twice with the above buffer and these supernatants were also collected and pooled. ^{125}I-labelled CgTx or [^{3}H]PN200-110 binding sites remaining in the supernatant after immunoprecipitation were precipitated by polyethylene glycol[29]. The total amount of labelled Ca^{2+} channel in assays was defined as the sum of the specifically bound c.p.m. immunoprecipitated plus the remaining specifically bound c.p.m. precipitated by polyethylene-glycol from the supernatant.

positive voltages, can mediate both transient and sustained Ca^{2+} currents, and are inhibited potently by ω-conotoxin[14]. L-type Ca^{2+} channels are also activated at relatively positive voltages, mediate long-lasting Ca^{2+} currents, and are inhibited potently by dihydropyridines and only weakly by ω-conotoxin[15,16]. The monoclonal antibody MANC-1 recognizes the α2 subunit of skeletal muscle dihydropyridine-sensitive L-type Ca^{2+} channels and immunoprecipitates 95.3 ± 1.3% of dihydropyridine-sensitive Ca^{2+} channels from rabbit skeletal muscle that have been prelabelled with [^{3}H]PN200-110. It also immunoprecipitates 65% of the L-type Ca^{2+} channels labelled with [^{3}H]PN200-110 from rabbit brain, and analysis of the immunoprecipitates reveals α1, α2, and β-like subunits of brain L-type Ca^{2+} channels[17]. To determine whether MANC-1 specifically recognizes L- or N-type Ca^{2+} channels in the rabbit hippocampus, Ca^{2+} channels in synaptic plasma membranes from this region were prelabelled with [^{3}H]PN200-110 or [^{125}I]ω-conotoxin (CgTx), solubilized with digitonin, and immunoprecipitated with the antibody. MANC-1 immunoprecipitated 82.5 ± 7.8% ($n = 6$) of the dihydropyridine-sensitive, L-type Ca^{2+} channels from synaptic plasma membrane preparations prelabelled with [^{3}H]PN200-110; preimmune mouse IgG immunoprecipitated 3.4 ± 1.7% (Fig. 1). In contrast, only 6.9 ± 2.0% ($n = 4$) of the specifically bound ^{125}I-labelled CgTx was immunoprecipitated by MANC-1 (IgG, 0.9 ± 0.6%). Most of this small amount of specifically bound ^{125}I-labelled CgTx that is immunoprecipitated probably

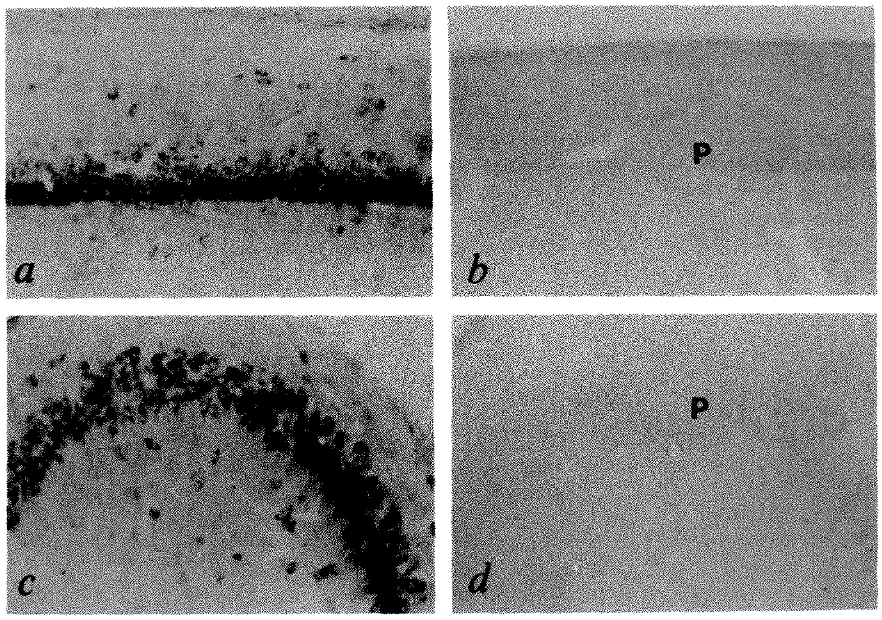

FIG. 2 Localization of L-type voltage-sensitive Ca^{2+} channels in hippocampal pyramidal neurons and interneurons. *a*, Sagittal section of CA1 region stained with MANC-1 (magnification ×102). *b*, Control section of CA1 region illustrating lack of staining, especially the pyramidal layer (P), when MANC-1 was preincubated with purified Ca^{2+} channels before tissue staining (magnification ×102). Sagittal section of CA2–CA3 region demonstrating neurons stained with MANC-1 in strata oriens, pyramidale (P) and radiatum (magnification ×90). *d*, Control section of CA1 region (magnification ×102).

METHODS. Adult Sprague-Dawley rats ($n = 17$) were anaesthetized with sodium pentabarbitol and intracardially perfused with solution of 4% paraformaldehyde[30]. The brains were immediately removed from the cranium, postfixed overnight, successively sunk in 10% (8 h), 20% (12 h) and 30% (48 h) solutions (w/v) of sucrose in phosphate buffer[30] at 4 °C and then sagittal or coronal (35 μm) sections were cut on a sliding microtome. Using the indirect peroxidase-antiperoxidase method, free floating sections were processed for immunocytochemistry as previously reported[30]. All solutions were the same except 0.1% Triton X-100 was replaced by 0.1% digitonin. In brief, sections were incubated in the following antisera: 1, MANC-1 IgG purified by chromatography on Protein-A Sepharose and diluted 1:20, for 36 h at 4 °C; 2, goat anti-mouse IgG diluted 1:30 for 1 h at 37 °C; and 3, mouse PAP diluted 1:100 for 1 h at 37 °C. Sections were then reacted with 0.4% 3,3′-diaminobenzidine tetrahydrochloride and 0.003% H_2O_2 and then mounted, dehydrated, covered with a coverslip and viewed. Control sections included replacing primary antisera with either normal mouse serum or buffer, and preincubating the MANC-1 antibody with purified skeletal muscle Ca^{2+} channel.

represents low affinity binding to L-type Ca^{2+} channels[14]. Thus, these results indicate that MANC-1 specifically recognizes dihydropyridine-sensitive L-type Ca^{2+} channels in the hippocampus.

Immunocytochemical studies using MANC-1 reveal that L-type Ca^{2+} channels are present in neurons located throughout the entire hippocampal region. Figure 2*a* and *c* illustrates cell bodies of pyramidal neurons and interneurons stained with MANC-1 in the strata oriens, pyramidale, and radiatum of the CA1 and the CA2–CA3 regions. Regions of the hippocampus containing axonal tracts are unstained. As a control, MANC-1 was preabsorbed with purified skeletal muscle Ca^{2+} channel before incubation with the tissue sections; MANC-1 staining was completely absent (Fig. 2*b*, *d*). Similar results are obtained in other control sections where MANC-1 was replaced by normal mouse serum or buffer.

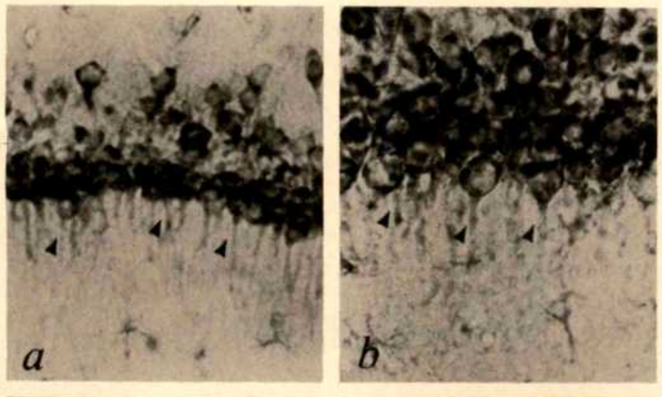

FIG. 3 Cellular distribution of L-type Ca^{2+} channels on hippocampal pyramidal neurons. *a*, *b*, Transverse sections of CA1 and CA2–CA3, respectively, stained using MANC-1. These panels illustrate L-type Ca^{2+} channels in the cell bodies and proximal portions of apical dendrites (arrowheads) of hippocampal pyramidal cells (*a*, ×448; *b*, ×384). *c*, *d*, Sagittal sections of CA1 and CA2–CA3, respectively. Dense MANC-1 staining is observed at the base of apical and basal dendrites (arrowheads) and in the proximal portion of apical dendrites (*c*, ×246; *d*, ×266). *e*–*g*, Higher magnifications of individual neurons illustrating dense immunoreactivity at base of dendrites (arrowheads) and around the periphery of the soma (*e*–*g*, ×1,100).

Dendrites immunoreactive for L-type Ca^{2+} channels are observed associated with both pyramidal neurons and interneurons (Fig. 3). The apical dendrites of pyramidal neurons located in the CA1 and in the CA2–CA3 regions that stain with MANC-1 are most distinct when viewed in coronal sections (Fig. 3*a*, *b*, arrowheads), but are also observed in sagittal sections (Fig. 3*c*, *d*). When viewed in either coronal or sagittal sections, MANC-1 staining only extends along the proximal portion of these dendrites. Measurements made from the centre of the cell body indicate that MANC-1 immunoreactivity extends 17–50 μm (mean = 35 μm, $n = 30$) along the apical dendrites of CA1 and CA2–CA3 cells. These results suggest a discrete localization of these L-type Ca^{2+} channels in the cell bodies and proximal dendrites of hippocampal pyramidal cells.

Close examination of the pattern of immunostaining of the cell bodies of solitary pyramidal cells reveals that the L-type Ca^{2+} channels recognized by MANC-1 are clustered at the base of the apical and basal dendrites of the pyramidal cells (Fig. 3*c*, *d*, arrowheads). At higher magnifications (Fig. 3*e*–*g*), L-type Ca^{2+} channels seem to be located in a thin band around the periphery of the cell soma with relatively denser staining at sites where basal and apical dendrites originate from the cell body (arrowheads). These regions of clustering of L-type Ca^{2+} channels are generally located 5–10 μm ($n = 30$) from the centre of the cell body. Immunoreactivity for L-type Ca^{2+} channels is always observed along the proximal region of the apical dendrites, but does not usually extend for a measurable distance along the basal dendrites (Fig. 3*a*–*g*).

Previous physiological studies have shown that Ca^{2+} channels that mediate long-lasting, high threshold Ca^{2+} currents are present in the dendrites[1,18] and in the somata[19] of hippocampal neurons. Our results with an antibody that recognizes 82.5% of L-type Ca^{2+} channels in the hippocampus define the distribution of these L-type channels on the surface of both the cell body and the proximal dendrites of CA1–3 pyramidal neurons. These channels are expected to contribute to the high threshold Ca^{2+} currents measured in these regions. The high density of L-type Ca^{2+} channels at the base of dendrites suggests that they may mediate large local Ca^{2+} currents at that site. L-type Ca^{2+} channels have also been detected in the dendritic fields of the hippocampal pyramidal cells by receptor autoradiography with radiolabelled dihydropyridines[20,21], and N-type Ca^{2+} channels have been observed on the cell bodies of individual hippocampal neurons in cell culture with fluorescent derivatives of ω-CgTx (ref. 22). N-type Ca^{2+} channels and L-type Ca^{2+} channels that are not recognized by our antibody may be localized on distal dendrites and may contribute to the Ca^{2+}-dependent action potentials and intracellular Ca^{2+} accumulation observed there.

The localization of L-type Ca^{2+} channels in proximal dendrites of the pyramidal cells and the clustering of L-type Ca^{2+} channels at the base of their major dendrites are provocative findings in light of recent observations on the role of voltage-gated Ca^{2+} channels in intracellular calcium transients. The spatial distribution of intracellular Ca^{2+} accumulation in CA1 pyramidal cells induced by stimulation of the distal dendrites has a marked peak in the proximal dendrites[10]. These results led to the suggestion that voltage-gated Ca^{2+} channels may be unevenly distributed along dendrites, with a higher density of Ca^{2+} channels in proximal regions[10]. Our results provide direct evidence for such an uneven distribution of L-type Ca^{2+} channels and, in addition, show that these channels are clustered at the base of the dendrites of the pyramidal cells. We conclude that L-type Ca^{2+} channels that we have observed may be responsible for the sharp peak in Ca^{2+} accumulation in the proximal dendrites[10]. Other types of voltage-gated Ca^{2+} channels that are not recognized by our antibodies may also contribute to the sharp peak of Ca^{2+} accumulation in the proximal dendrites because the distribution of L-type Ca^{2+} channels described here does not precisely overlap the previously recorded intracellular Ca^{2+} transients[10]. However, when comparing our findings with those from Ca^{2+} imaging[10] surface-to-volume ratios must also be taken into consideration. In the cell body, entering Ca^{2+} is diluted into a large internal volume relative to surface area. In contrast, proximal dendrites have a much smaller internal volume relative to their surface area. For an equal rate of Ca^{2+} entry, the increase in cytosolic Ca^{2+} will be much larger in the proximal dendrites than in the soma. Similarly, because the diameter of the dendrites is smaller at more distal sites, the peak of cytosolic Ca^{2+} should be displaced to a more distal position than the peak of Ca^{2+} channel density, as we have observed. Thus, the distribution of L-type Ca^{2+} channels in the proximal dendrites and soma is consistent with a causal role in mediating cytosolic Ca^{2+} transients in response to stimulation of the distal dendritic tree.

Calcium entry through the NMDA-specific class of excitatory amino-acid receptors in synapses on distal dendrites induces long-term potentiation in CA1 pyramidal cells[8,23-25]. We suggest that Ca^{2+} entry through L-type Ca^{2+} channels clustered in proximal dendrites and soma is activated by these same synaptic inputs and serves to initiate other intracellular regulatory events. These events may include Ca^{2+}-dependent protein phosphorylation and activation of gene expression. Thus, these strikingly localized L-type voltage-gated Ca^{2+} channels may serve as critical elements in neural integration and signal processing at the single cell level. □

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Anion channels activated by adrenaline in cardiac myocytes

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In heart cells, the catecholamine-activated cyclic AMP system regulates calcium[1] and potassium[2-4] channels. We report here a novel class of chloride channels that can be activated by adrenaline in mammalian ventricular cells. Like the agonist-activated Cl^- channel currents of airway[5-9] and colonic[10,11] epithelial cells, the cardiac Cl^--channel current shows outward rectification. But the unit conductance of cardiac Cl^- channels is smaller than that of epithelial Cl^- channels. The cardiac Cl^- channel is functionally voltage-independent, in contrast to the Cl^- channel in colonic epithelial cells[10,11]. This channel could be responsible for the β-catecholamine-induced increase in cardiac membrane conductance[12] that has been attributed to activation of a Cl^- current[13-15]. Thus, sympathetic control of cardiac electrical activity involves not only the voltage-dependent, excitation-related cation channels, but also anion channels that generate a steady current.

Activation of channel currents in a cell-attached patch was observed when adrenaline was added to the bath (Fig. 1*a*). The patch membrane potential was clamped at a voltage 40 mV more negative than the resting potential (RP −40 mV). Compared to the usual delay of about 15 s for activation, the channel currents were deactivated more slowly by removing adrenaline, which seems consistent with involvement of intracellular metabolic pathways. We observed such adrenaline-activated channel currents in less than 5% of cell-attached patch recordings. The low success rate, which limited the number of cases analysed, is partly due to low channel density (see below).

Like other catecholamine-dependent ion channels in heart cells[1-4], the channels seemed to be regulated by cyclic AMP. Thus, channel currents with conductance and kinetics similar to those activated by adrenaline can be induced by exposing the cell to a high concentration (0.5 mM) of dibutyryl cAMP (db-cAMP). Figure 1*b* shows an example of such currents.

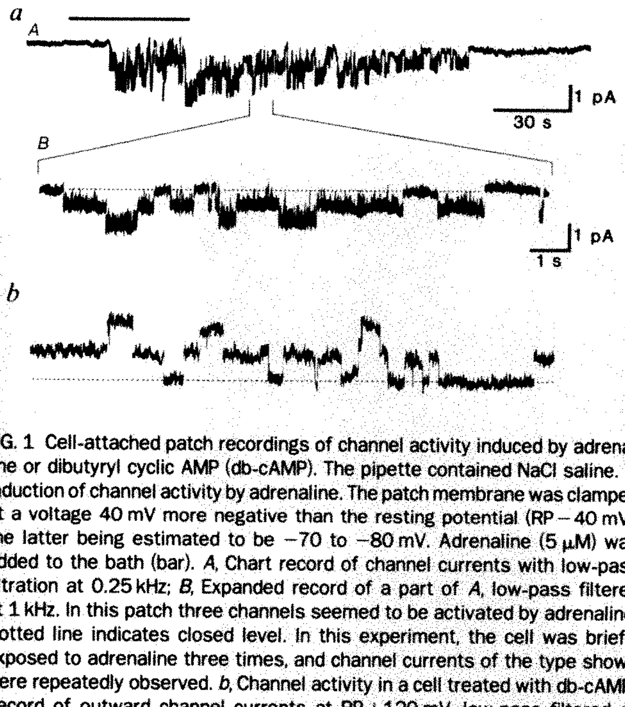

FIG. 1 Cell-attached patch recordings of channel activity induced by adrenaline or dibutyryl cyclic AMP (db-cAMP). The pipette contained NaCl saline. *a*, Induction of channel activity by adrenaline. The patch membrane was clamped at a voltage 40 mV more negative than the resting potential (RP − 40 mV), the latter being estimated to be −70 to −80 mV. Adrenaline (5 μM) was added to the bath (bar). *A*, Chart record of channel currents with low-pass filtration at 0.25 kHz; *B*, Expanded record of a part of *A*, low-pass filtered at 1 kHz. In this patch three channels seemed to be activated by adrenaline. Dotted line indicates closed level. In this experiment, the cell was briefly exposed to adrenaline three times, and channel currents of the type shown were repeatedly observed. *b*, Channel activity in a cell treated with db-cAMP. Record of outward channel currents at RP + 120 mV, low-pass filtered at 0.5 kHz. Two channels were active in this patch. Calibration in *aB* also applies to *b*. In this type of experiment, cells were exposed to 0.5 mM db-cAMP for at least 10 min before formation of the patch. In three out of 71 such patches, channel activity was detected.

METHODS. Single cells were isolated from guinea-pig heart ventricles using collagenase[28,29]. Single channel currents were recorded from cell-attached membrane patches[30]. The patch electrode had a resistance of 3–5 MΩ when filled with pipette solution. The bath solution was Tyrode solution containing NaCl 140 mM, KCl 5.4 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1 mM, glucose 5.5 mM and HEPES-NaOH 5 mM (pH 7.4). Adrenaline (1–5 μM) and db-cAMP (0.5 mM) were applied to the cells by adding these agents to the bath solution. Three types of pipette solution were used. The NaCl saline contained NaCl 140 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1 mM and HEPES-NaOH 5 mM (pH 7.4). This solution also contained 2 mM $BaCl_2$ to block K^+ channels. The high-NaCl saline was prepared by adding 150 mM extra NaCl to the NaCl saline. The low-Cl $NaNO_3$ saline was made by replacing 130 mM NaCl in the NaCl saline with 130 mM $NaNO_3$. Experiments were performed at 34–36 °C.

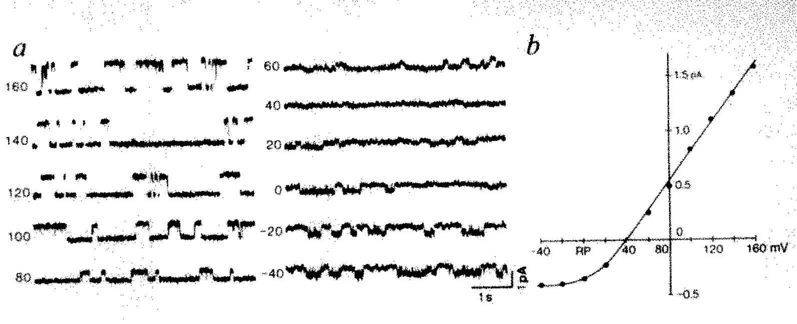

FIG. 2 Adrenaline-activated Cl^- channels at various membrane potentials in a cell-attached patch. The patch membrane potential was varied while adrenaline-induced channel activity was seen. The pipette contained NaCl saline (149.6 mM Cl^-). *a*, original records of single channel currents, filtered at 0.5 kHz. Adrenaline concentration, 5 μM. Numbers at the left of each trace indicate deviation (in mV) from the resting potential. *b*, relationship between amplitude of single channel current (ordinate) and membrane potential (abscissa), obtained from the data shown in *a*. Membrane potentials are expressed as in *a*. The single channel conductance for the outward currents was 13 pS, and the reversal potential was RP + 40 mV.

Unlike the inward currents, outward currents through these channels showed no bursting activity.

Figure 2 shows original recordings of the adrenaline-activated channel currents and their I-V relation. The I-V relation of the outward currents seemed to be linear, but the inward currents showed a trend of saturation with hyperpolarizations (outward rectification). The single-channel conductance for outward currents was 13.1 ± 1.2 pS (mean ± s.d., $n = 4$), and the reversal potential, estimated from I-V relation, was RP + 37 ± 4 mV. The outward rectification was generally present, but its degree was variable (see also Fig. 3*c*). If the cell membrane potential during adrenaline application is −80 to −70 mV[12], the absolute reversal potential of channel currents may be −43 to −33 mV. These values do not match the equilibrium potential of any principal cations present inside and outside the cells, but appear to be close to the Cl^- equilibrium potential (−60 to −40 mV) calculated using the reported cardiac intracellular Cl^- activity[16-20].

Cl^- selectivity of the channel was further tested by nearly doubling the NaCl concentration in the pipette. If the channel is Cl^- selective, a more negative reversal potential and an increased outward channel conductance should be recorded, whereas a positive shift of the reversal potential should occur for Na- or cation-selectivity. The reversal potential obtained with the high-NaCl saline (estimated to be RP + 4 mV) was more negative than that obtained before, and the conductance for outward currents was increased to 20 pS (Fig. 3*a*, *c*). In two similar experiments, the reversal potential was RP +25 and RP +21 mV, and the conductance was 16 and 17 pS. We conclude that the adrenaline-activated channel is Cl^- selective. The variation in the reversal potential may be attributed to variations in the resting potential and/or variations of intracellular Cl^- concentration in individual cells. When the pipette contained low-Cl^- $NaNO_3$ saline (Fig. 3*b*, *c*), channel activities similar to those obtained with NaCl saline were observed. NO_3^- ions have been suggested to support anion currents in cardiac tissue[21,22] as well as in other excitable tissues[23].

The channel showed characteristic kinetics. The open-time histogram of outward currents was fitted to a single exponential function with a long mean open-time of 200–700 ms ($n = 4$, Fig. 4*a*). The inward currents showed high-frequency closings during the burst (Figs 1*a*, 2*a* and 3*a*, *b*), indicating presence of an additional fast-gating process or some blocking phenomena[23]. The mean burst-time, obtained for inward currents by neglecting the short (<2 ms) closings, was in the same order as the mean open-time for outward currents. Although different in different patches, the mean open-time and burst-time, and open probability (P_0) (Fig. 4*b*), showed no systematic variation with voltage.

Our results provide direct evidence, for intact cardiac membrane, that β-adrenoceptor-activated Cl^- current recorded under whole-cell conditions[13-15] flows through ion channels

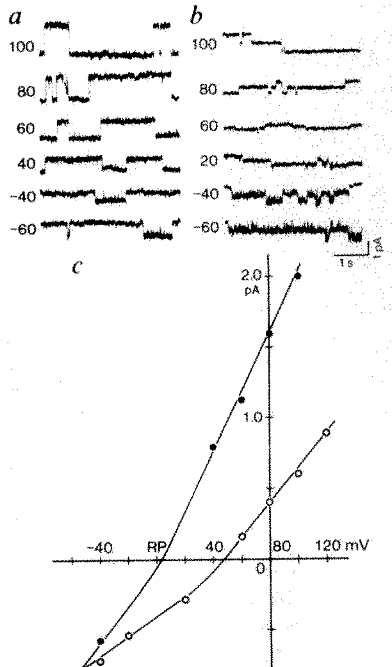

FIG. 3 Adrenaline-activated Cl^- channels with different pipette solutions. The pipette contained either high-NaCl saline (299.6 mM Cl, *a*) or low-Cl $NaNO_3$ saline (130 mM NO_3 + 19.6 mM Cl, *b*). *a* and *b*, Original records of single channel currents at various membrane potentials, filtered at 1 kHz. Adrenaline concentration, 5 μM. Two channels were active in *b*. *c*, I-V relation of the channel current obtained with high-NaCl saline (●) and low-Cl $NaNO_3$ saline (○). Details are as in Fig. 2.

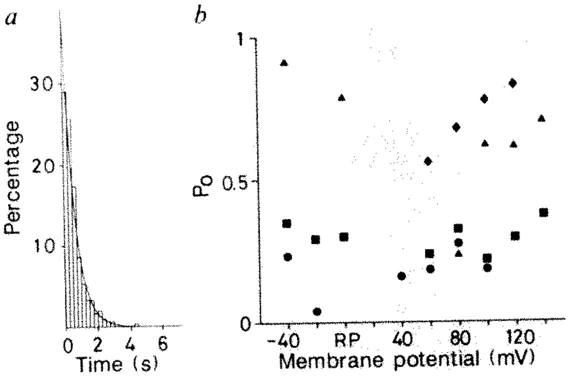

FIG. 4 *a*, Open-time histogram obtained at RP + 120 mV. Number of events sampled was 298. Time bins are 250 ms. The histogram was fitted to a single exponential with a time constant of 637 ms. The channel was activated by 5 μM adrenaline. *b*, Open probability of the channel (P_0, calculated as a fraction of time for which the channel stays open), as a function of membrane potential (abscissa). Membrane potentials are expressed as amounts of deviation from the resting potential (RP). The channels were all activated by 5 μM adrenaline. P_0 was calculated from the data filtered at 1 kHz. For −40 to 0 mV, at which potentials the currents were inward and showed high-frequency closings during the burst, closing gaps shorter than 2 ms were neglected for calculation. Data from four patches (different symbols).

rather than an electrogenic cotransporter[24]. Unitary Cl$^-$ currents have been recorded in cardiac membrane vesicles incorporated into lipid bilayers[25]. But the vesicular Cl$^-$ channels are active without catecholamine and their conductance is much larger (55 pS), suggesting a different kind of Cl$^-$ channel. The cardiac Cl$^-$ channels reported here have properties different from the agonist-activated Cl$^-$ channels in epithelial cells. The unit conductance of cardiac Cl$^-$ channels is smaller than that of epithelial Cl$^-$ channels (25 to 50 pS)[5-11]. The cardiac Cl$^-$ channel is practically voltage-independent, in contrast to the Cl$^-$ channel in colonic epithelial cells[10,11]. Detailed voltage-dependence is not known in the Cl$^-$ channels of other types of epithelial cells.

In our previous study[15], adrenaline increased membrane conductance by 10 nS in a whole cell. If the open probability of the channel is assumed to be 0.5, the 10 nS increase in conductance is produced by about 1,600 channels. This leads to estimation of a low channel density (about 0.1 μm^{-2}) using average cell surface area of 14,000 μm^2, the latter value being obtained from capacitance measurements of ventricular cells[15,26] with an assumption of specific membrane capacitance of 1 μF cm^{-2}.

Activation of the anion channel by β-adrenergic stimulation should accelerate action potential repolarization in cardiac muscle, associated with increased heart rate. The Cl$^-$ channel may also be involved in the natural pacemaker mechanism. The channel activated by catecholamine should increase background inward currents at the diastolic potential level, which would produce an accelerated pacemaker rhythm.

As whole-cell studies demonstrate that activation of cardiac Cl$^-$ current by β-adrenoceptor stimulation is mediated by cAMP-dependent protein kinase[13,14,27], it is likely that the same mechanism is involved in the activation of the anion-channel currents described here. Induction of similar channel currents by db-cAMP supports this view. If so, cAMP-dependent protein kinase regulates, in heart muscle membrane, not only voltage-dependent, excitation-linked cation channels such as the Ca^{2+} channel[1] and the delayed rectifier K$^+$ channel[2-4], but also anion channels that generate a steady current. Studies on excised patches should allow detailed investigation of intracellular regulatory mechanisms of these channels, with improved experimental control. □

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Death of mature T cells by separate ligation of CD4 and the T-cell receptor for antigen

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EFFECTOR T cells are restricted to recognizing antigens associated with major histocompatibility complex (MHC) molecules[1]. Specific recognition is mediated by the $\alpha\beta$ heterodimer of the T-cell receptor (TCR)/CD3 complex[2,3], although other membrane components are involved in T-cell antigen recognition and functions. There has been much controversy in this regard over the part played by the CD4 glycoprotein. It is known that expression of CD4 correlates closely with the cell's ability to recognize antigens bound to class II MHC molecules[4,5] and that CD4 can bind to class II molecules[6]. Also monoclonal antibodies to CD4 can modify signals generated through the TCR/CD3 complex[7-10]. It has therefore been proposed that CD4 binds to class II molecules, coaggregates with the TCR–CD3 complex and aids the activation of T cells. But given that TCR can itself impart restriction on the cell, it remains unclear whether the contribution of CD4-derived signals to those generated through the TCR$\alpha\beta$–CD3 complex is central to this activation. Here we report that when preceded by ligation of CD4, signalling through TCR$\alpha\beta$ results in T cell unresponsiveness due to the induction of activation dependent cell death by apoptosis. These results imply that CD4 is critically involved in determining the outcome of signals generated through TCR, and could explain why the induction of effector T cells needs to be MHC-restricted.

Monoclonal antibodies to TCR$\alpha\beta$ (H57.597, ref. 11) stimulated DNA synthesis by splenic T cells bearing the CD4 antigen in the presence of T cell-depleted irradiated splenocytes. The level of thymidine incorporation induced by the antibodies was maximal over a broad concentration range (Fig. 1), titering to background between 1–10 ng ml^{-1}. Pretreatment with antibodies to CD4 inhibited the ability of the anti-TCR$\alpha\beta$ antibodies to induce DNA synthesis (Fig. 1). Two monoclonal antibodies to CD4 (H129, ref. 12; GK 1.5, ref. 13) had the same effect and both required crosslinking with polyclonal antiserum (data not shown).

By contrast, the response to antibodies to CD3ε (145.2C11, ref. 14) was virtually unaffected by pretreatment with anti-CD4 antibodies (Fig. 1). The maximum inhibition was 40%, occurring only at the lowest concentration of the stimulating antibody (Fig. 1), and the level of thymidine incorporated was consistently higher than that induced by antibodies to TCR$\alpha\beta$. This differential capacity to signal through TCR$\alpha\beta$ and CD3ε has been reported previously in other systems[15] and may reflect either differences in antibody affinities or a functional uncoupling of TCR$\alpha\beta$ from CD3 components[15,16]. The differential effects of anti-CD4 antibodies in perturbing signals generated through TCR$\alpha\beta$ and CD3ε supports the latter hypothesis (see below). To determine whether membrane-associated molecules other than CD4 could alter signals generated through TCR$\alpha\beta$, we analysed the effects of crosslinking MHC class I, LFA-I and CD2 to TCR$\alpha\beta$-CD3 by treatment with the appropriate antibodies. Cell surface expression of these molecules was easily detectable by flow cytometry (data not shown). Crosslinking did not affect levels of thymidine incorporation induced either by anti-TCR$\alpha\beta$ or anti-CD3ε antibodies (Table 1).

The inability to induce DNA synthesis through TCR$\alpha\beta$ on cells pretreated with anti-CD4 antibodies was not due to

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TABLE 1 Specificity of CD4-mediated inhibition

	% of control response after pretreatment with antibodies against:			
Stimulus	CD4	MHC class I	LFA-1	CD2
Anti-TCR$\alpha\beta$	13	101	100	93
Anti-CD3ε	109	148	112	113

T cells carrying the CD4 antigen were prepared as described in Fig. 1. Cells were pretreated at 10^7 ml^{-1} with either anti-CD4 (H129), anti-MHC class I (M142, ref. 32), anti-CD2 (12-15, ref. 33), or anti-LFA-1 (I21/7.7, ref. 34) each at 50 µg ml^{-1} for 45 min at room temperature, as described in Fig. 1. Treated cells were cultured with the indicated stimuli at 100 ng ml^{-1} and collected as described in Fig. 1.

comodulation of TCR-CD3 with CD4[17,18]. Levels of TCR$\alpha\beta$ expression, determined flow cytometrically, were unaffected by pretreatment with anti-CD4 antibodies (data not shown). The pretreatment did not alter the ability of anti-TCR$\alpha\beta$ antibodies to induce increases in intracellular free Ca^{2+} concentrations, and neither the frequency of responding cells nor the amplitude of the induced Ca^{2+} signal was affected (data not shown). The inhibition of DNA synthesis and the lack of inhibition of an early signalling event suggests that pretreatment with antibodies to CD4 alters, rather than ablates, signalling through TCR$\alpha\beta$.

Apoptosis is a form of cell death characterized by the generation of nucleosome-sized fragments as a result of the activation of an endogenous endonuclease that requires Ca^{2+} (ref. 19). Apoptosis mediated by TCR-CD3ε occurs in thymocytes[20-22], as well as in hybridomas derived from mature T cells[23]. As pretreatment with anti-CD4 antibodies does not inhibit the ability of anti-TCR$\alpha\beta$ to induce mobilization of intracellular Ca^{2+}, we addressed the possibility that cells pretreated with anti-CD4 antibodies die when stimulated through TCR$\alpha\beta$.

The induction of apoptosis was demonstrated by visualization of nucleosome-sized DNA fragments[19] and quantitated by assessing the proportion of fragmented DNA[24]. Overnight culture of T cells bearing the CD4 antigen with anti-TCR$\alpha\beta$ antibodies under crosslinking conditions induced a <5% increase in the level of fragmented DNA over the background level observed in the absence of anti-TCR$\alpha\beta$ antibodies (Fig. 2, lanes 1 and 2; Fig. 3). As previously reported for immature thymocytes[21], anti-CD4 crosslinking in the absence of subsequent stimulation did not give levels of DNA fragmentation (9.4 ± 2.8%) significantly different from those obtained on stimulation

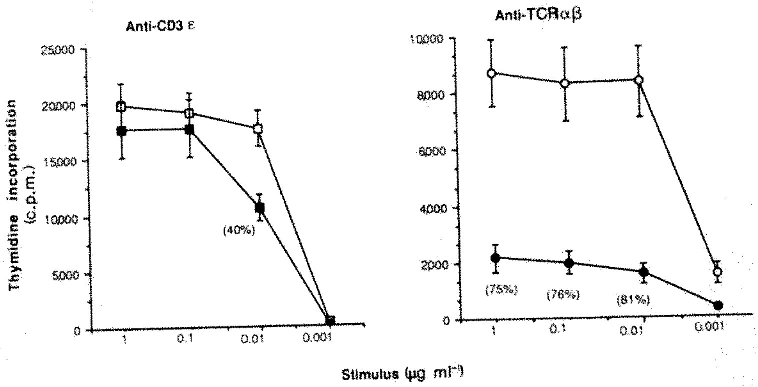

FIG. 1 CD4-mediated inhibition of anti-TCR$\alpha\beta$ and anti-CD3ε induced stimulation. The stimulation of CD4$^+$ splenic T cells was quantitated by measuring antibody-mediated [^{3}H]thymidine incorporation by T cells (3×10^4) in the presence of irradiated T cell-depleted spleen cells (5×10^5). Splenic CD4$^+$ T cells were prepared from BALB/c mice (Charles River, Montreal, Quebec). B cells were depleted by passage of splenocytes over immunoglobulin-anti-immunoglobulin columns (Beckman) and the resulting population treated with anti-CD8 antibody (3.168, ref. 35) plus complement. The remaining viable cells were separated on discontinuous percoll gradients. Those cells binding at the $\rho = 1.109/1.079$ interface were used throughout. These cell preparations were consistently >95% positive for Thy-1.2, TCR-CD3 and CD4. They were <1% positive for mIg, class II and CD8. Filler cells were prepared by treating syngeneic splenocytes simultaneously with anti-Thy-1.2, anti-CD4 and anti-CD8 in the presence of complement. Viable cells were isolated on discontinuous percoll gradients ($\rho = 1.109/1.066$) before gamma irradiation (2,000 rad). T cells carrying the CD4 antigen were pretreated or not at 10^7 ml^{-1} with 50 µg ml^{-1} H129 or GK1.5 for 45 min at room temperature, washed, and bound antibodies crosslinked with rabbit anti-mouse immunoglobulin at 50 µg ml^{-1} for 45 min at 37 °C. Following this treatment the cells were washed and plated with fillers and the indicated concentrations of 145.2C11 and H57.597. After 36 h of culture in Iscove's modified Dulbecco's medium (IMDM) with no serum supplements, each culture was pulsed with 1 µCi of [^{3}H]thymidine and collected 6 h later. Numbers represent the mean and one standard error from a pool of 8 individual experiments. Numbers in parenthesis, where indicated, represent per cent inhibition compared with non-pretreated controls. Left panel, Anti-CD3ε (145.2C11) induced thymidine incorporation before (□) and after (■) anti-CD4, rabbit anti-mouse immunoglobulin pretreatment. Right panel, Anti-TCR$\alpha\beta$ (H57.597) induced thymidine incorporation before (○) and after (●) anti-CD4 rabbit anti-mouse immunoglobulin pretreatment.

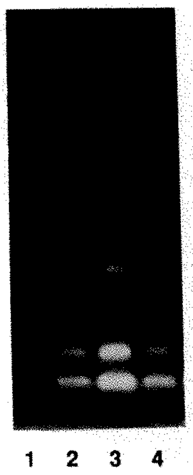

FIG. 2 Induction of apoptosis in peripheral CD4$^+$ T cells. T cells carrying the CD4 antigen, pretreated or not as described in Fig. 1, were incubated at 10^7 ml^{-1} with anti-TCR$\alpha\beta$, anti-CD3ε, and in some cases simultaneously with anti-CD4, each at 10 µg ml^{-1} for 45 min at room temperature. After washing, 1.3×10^6 cells were plated in 0.5 ml IMDM in 24-well Costar plates precoated with rabbit anti-mouse immunoglobulin at 50 µg ml^{-1}. After overnight incubation, the cells were collected and the extent of DNA fragmentation determined by a modification of the method of Sellins and Cohen[19]. In brief, the cells were collected by centrifugation at 200*g* for 10 min. The pellet was lysed with 0.5 ml hypotonic lysing buffer, pH 7.5 (10 mM Tris buffer, 1 mM EDTA, 0.2% Triton X-100). The lysates were immediately centrifuged at 13,000*g* for 10 min and the supernatant, containing fragmented DNA, was collected. DNA from the 13,000*g* supernatants were precipitated overnight at −20 °C in 50% isopropanol and 0.5 M NaCl. The precipitates were collected after centrifugation at 13,000*g*, air-dried, and resuspended in 10 mM Tris, 1 mM EDTA, pH 7.4. Loading buffer containing 15 mM EDTA, 2% SDS, 50% glycerol and 0.5% bromphenol blue was added to samples at 1:5 (v/v), and samples heated to 65 °C for 10 min. Electrophoresis was in 0.75% agarose for 3 h at 90 V. DNA was visualized by staining with ethidium bromide, and photographed. Lane 1, untreated cells; lane 2, anti-TCR$\alpha\beta$; lane 3, pretreatment with anti-CD4 followed by anti-TCR$\alpha\beta$; lane 4, simultaneous addition of anti-CD4 and anti-TCR$\alpha\beta$.

with antibodies to TCR-CD3. Simultaneous exposure to anti-TCR$\alpha\beta$ and anti-CD4 antibodies under these conditions resulted in a slight decrease in the proportion of fragmented DNA (Fig. 2, lane 4; Fig. 3). By contrast, when cells were pretreated with anti-CD4 antibodies and then incubated overnight with anti-TCR$\alpha\beta$ antibodies, DNA fragmentation increased significantly, reaching levels up to 10 times those induced by simultaneous addition of anti-TCR$\alpha\beta$ and anti-CD4 antibodies (Fig. 2, lane 3; Fig. 3). We conclude that CD4 plays a critical part in determining the outcome of signals generated through TCR$\alpha\beta$.

In contrast to the results obtained with anti-TCR$\alpha\beta$, addition of anti-CD3ε to cells pretreated with anti-CD4 did not increase the level of DNA fragmentation above that induced in the absence of anti-CD4 pretreatment (Fig. 3). These results suggest that TCR$\alpha\beta$ can be functionally uncoupled from some CD3 components. This could be explained if anti-TCR$\alpha\beta$ were to induce a series of progressive activation signals. The involvement of CD3 components in this progression could be preceded by several events which could be susceptible to CD4-mediated regulation. Those signals generated directly through some CD3 components, on the other hand, may not be. Hence, direct ligation of CD3ε with antibody could functionally obviate upstream signals generated through TCR$\alpha\beta$. Alternatively, or in addition, CD3 could exist as functionally distinct signalling complexes, perhaps generated as a consequence of TCR-CD3 interaction with CD4. Signalling through these CD3 components would circumvent the requirements for TCR$\alpha\beta$ ligation. Our results predict that unless CD4 is incorporated in the TCR-CD3 complex, the generation of signals through TCR$\alpha\beta$ will result in cell death. Why then is there any T-cell activation on direct ligation of TCR$\alpha\beta$ *in vitro*? The requirement for oligomerization of TCR$\alpha\beta$ to induce activation signals[15,16] could lead to the non-specific trapping of CD4 molecules. This inefficiency may account for the differential response to anti-TCR$\alpha\beta$ and anti-CD3ε antibodies in the absence of anti-CD4 pretreatment (Fig. 1).

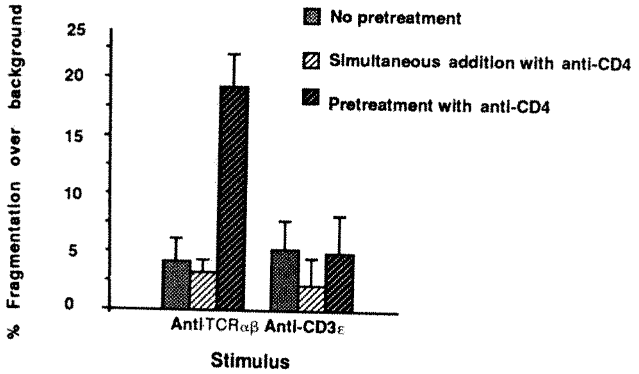

FIG. 3 DNA Quantitation. The DNA in the precipitates and supernatants generated from the 13,000g spin of lysed cells (Fig. 2, legend) was precipitated overnight at 4 °C in 0.5 ml of lysis buffer and 0.125 ml of 50% trichloroacetic acid, and centrifuged at 13,000g for 4 min. The DNA was hydrolysed by heating to 90 °C for 10 min in 240 μl 5% trichloroacetic acid and quantitated by a modification of the diphenylamine method of Burton[24]. In brief, 0.48 ml of DPA reagent [0.15 g DPA, 0.15 ml sulphuric acid, 0.05 ml acetaldehyde (16 mg ml^{-1})/10 ml glacial acetic acid] was added to each tube. After a 48-h incubation at 4 °C, absorbance at 570 nm was measured. Per cent fragmentation was calculated as the ratio of DNA in the 13,000g supernatants to the total DNA recovered in the supernatant and the pellet. Per cent fragmentation of immediately *ex vivo* CD4$^+$T cells was 10.2%. Data is presented as % fragmentation over background which averaged to 25.92 ± 5.5 in eight experiments reported. Crosslinking of anti-CD4 in the absence of subsequent stimulation resulted in an average increase of 9.4 ± 2.8% fragmentation over background.

The mechanism through which CD4 mediates its effect is not clear. Whether the requirement for crosslinking reflects the need to decrease the proximity of CD4 and TCR-CD3, or to sterically hinder their interaction, remains unclear. In addition, given that crosslinking CD4 results in the activation of the tyrosine kinase p56^{lck} (ref. 25), inappropriately directed kinase activity could also be involved. A precedent for the effects of separate ligation of CD4 and TCR$\alpha\beta$ has been established *in vivo*. Pretreatment of adult animals with anti-CD4 antibodies abrogates rejection of primary allografts as well as the production of alloantibodies[26,27]. Importantly, the results reported here are not easily reproducible using long-term propagated clones of CD4$^+$, MHC class II-restricted T cells MHJ (unpublished observation). Differences between signalling mediated by CD4 and TCR-CD3 in immediately *ex vivo* T cells and clones are becoming evident. For example, crosslinking of CD4 with antibody (ref. 28, and MHJ unpublished observations) on cloned, but not *ex vivo* (MHJ unpublished observations) T cells, results in Ca^{2+} mobilization. It is possible that signal transduction machinery in cloned T cells is irreversibly locked into those pathways which, in fact, reflect our ability to propagate these cells indefinitely.

The demonstration that CD4 binds MHC class II molecules[6], provides the mechanism through which it can interact with CD3 components associated with antigen/MHC class II-specific TCR molecules. Moreover, p56^{lck}, which physically associates with the cytoplasmic domain of CD4 provides a molecular basis through which the CD4 complex can modify CD3 components[29]. The ζ chain is present in the CD3 complex either as a covalently associated homodimer or as a heterodimer with the η chain[30]; experiments suggest that the consequences of signalling through each form is different[31]. Specifically, the ability to induce programmed cell death through TCR$\alpha\beta$ requires the presence of ζ-η heterodimers. On the basis of this and our results, one might speculate that association of CD4 with TCR-CD3 determines which CD3 components will function in TCR$\alpha\beta$-mediated signalling. Therefore, the fate of a T cell would be determined by the involvement of CD4 in antigen recognition, which would, in turn, explain why T cell activation need be MHC restricted. □

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Different amino acids at position 57 of the HLA-DQβ chain associated with susceptibility and resistance to IgA deficiency

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THE human leukocyte antigens (HLA) are implicated in the genetic susceptibility to a large number of diseases. Some of the diseases associated with HLA class II are related to specific amino acids or epitopes of the domain of the HLA class II molecule that is distal to the membrane[1–3]. In man, selective immunoglobulin A deficiency is the most common immunodeficiency, frequently resulting in recurrent sino-pulmonary infections and gastro-intestinal disorders. Associations have been described with HLA class I, and to a lesser extent with different class II alleles[4–9], which might indicate that they share some common feature. Here we study 95 IgA-D patients and find positive associations with three DR–DQ haplotypes and a strong negative association with a fourth haplotype. Comparison of the sequences of the polymorphic amino-terminal domain of the DQβ chain showed that the three 'susceptibility' haplotypes all had a neutral alanine or valine at position 57. The 'protective' allele had the negatively charged aspartic acid at this position (Asp 57). Codon 57 of the HLA-DQβ chain has been implicated in the susceptibility to insulin-dependent diabetes mellitus[1,2]. Our data suggest that the same amino acid position could possibly also influence susceptibility and resistance to selective immunoglobulin A deficiency.

First we confirmed the well-established DR3 association in selective immunoglobulin A deficiency (IgA-D). The DR3 specificity has recently been split and IgA-D was found to be associated with the DRw17 subtype. Positive associations with two additional DR-DQ haplotypes, DR7,DQw2 and DR1,DQw5 were observed, as well as a strong negative association with DRw15, DQw6 (Table 1). The increased relative risks (RR) conferred by the DRw17,DQw2 and DR7,DQw2 haplotypes were additive, whereas DR1,DQw5 acted synergistically with these two haplotypes: the RR of DRw17,DQw2/DR7,DQw2 heterozygous individuals being 8.9, whereas the RRs of DRw17, DQw2/DR1,DQw5 and DR7,DQw2/DR1,DQw5 heterozygotes were 37.3 and 17.2, respectively. This finding might indicate heterogeneity in the genetic susceptibility to IgA-D. Only 2 of 35 DR1,DQw5 positive IgA-D patients had anti-IgA antibodies (determined by haemagglutination) compared with 16 of 45 DRw17,DQw2 positive patients ($P < 0.005$), which supports this hypothesis.

Forty-seven IgA-D patients were HLA-DP typed by *Msp*I restriction fragment length polymorphism (RFLP) analysis[10]. No HLA-DP association was observed. The linkage disequilibrium between DRw17,DQw2 and DPw1 was not more pronounced in IgA-D than in controls. These findings suggest that the haplotypes associated with IgA-D are not extended centromerically throughout the entire HLA class II region. The associations with the HLA class I alleles B8 and B14 (refs 4–6) and with loci in the class III region[11] might indicate that the haplotypes associated with IgA-D are extended telomerically.

When looking for a shared feature between the observed DR–DQ associations, the published sequences of the polymorphic second exon of *DRB1*, *DQA1* and *DQB1* genes were compared. Analysis of *DRB1* and *DQA1* sequences were unrewarding. As can be seen in Table 2, comparison of the polymorphic residues of the HLA-DQβ chain in the IgA-D associated alleles were more suggestive. The three susceptibility alleles all had a neutral amino acid at position 57, alanine or valine, whereas the protective DRw15,DQw6 had the negatively charged aspartic acid. In addition, three *DQB1* alleles with neutral to negative associations with IgA-D were Asp 57 positive; **0301*, **0402* and **0603*. The importance of position 57 is further stressed by the observation that when individuals carrying the three DR–DQ haplotypes positively associated with IgA-D are removed from the patient group and the control group, 10 of the remaining 14 IgA-D patients are non-Asp 57 positive compared with 24 of the remaining 57 controls ($P < 0.05$). In the whole material, 62% of patients were HLA-DQβ non-Asp 57 homozygous compared with 22% of controls ($P < 10^{-7}$; RR 5.9). Only 4% of patients were Asp 57 homozygous compared with 34% of controls ($P < 10^{-5}$; RR 0.1) (Fig. 1). The

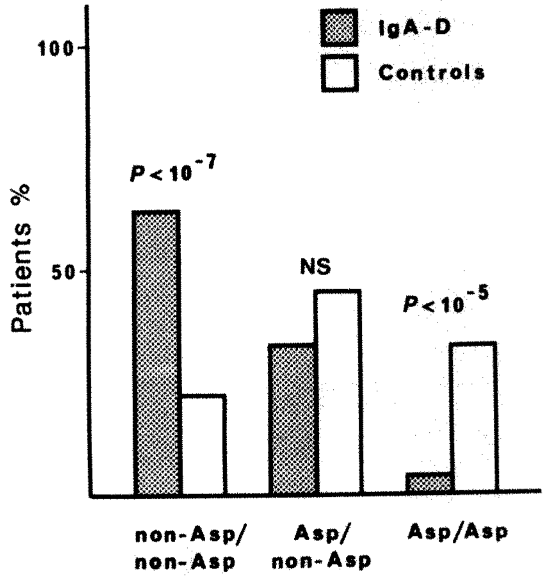

FIG. 1 The frequency of occurrence of Asp at position 57 of the HLA-DQβ chain in IgA-D patients compared with controls.

METHODS. All patients and controls were HLA-DR and -DQ typed by *Taq*I RFLP analysis[23–25] and the amino acid at position 57 was inferred. In 72 of the 95 patients and 94 of the 100 controls Asp 57 and non-Asp 57 was confirmed by hybridizing the second exon of *DQB1* genes[1] (amplified by polymerase chain reaction) with 8 oligonucleotide probes centred around codon 57 (ref. 13). The two methods gave completely concordant results.

TABLE 1 Phenotype frequencies (%) of HLA-DR-DQ haplotypes in IgA-D patients* and healthy Swedish controls

Dw†	DR†	DQ†	Phenotype frequency IgA-D (n=95)	Controls (n=100)	P_c‡	IgA-D association	Relative risk
1	1	w5	37	21	<0.005	positive	2.2
2	w15	w6	3	30	<0.001	negative	0.1
3	w17	w2	46	15	<0.0001	positive	4.9
17	7	w2	21	7	<0.02	positive	3.3

* IgA-D was defined as a serum level of IgA of less than 0.05 g l^{-1}. The IgA-D patients were unrelated and of Scandinavian extraction. One third of the patients had symptomatic IgA-D, in most cases manifested by recurrent sino-pulmonary infections.

† HLA-DR and -DQ typing was performed by *Taq*I RFLP analysis. For details of methodology and probes used see ref. 22. The serologically (DR and DQ) and cellularly (Dw) defined specificities associated with specific *Taq*I RFLP patterns[23-25] are given in the text and tables.

‡ Data were analysed by the χ^2 test or Fisher's exact test when appropriate. When looking for class II associations other than the well-established DRw17 association[5,7,8], gene frequencies of patients and controls were compared after subtraction of DRw17,DQw2 positive haplotypes. P values were corrected (P_c) for multiple comparisons.

TABLE 2 Comparison of HLA-DQβ chain first domain polymorphic amino-acid residues in IgA-D-associated DQ alleles

Dw	DR	DQ	*DQB1*	IgA-D-assoc.	10	20	30	40	50	60	70	80	90	
3	w17	w2	**0201*	positive	RDSPEDFVYQ	FKGMCYFTNG	TERVRLVSRS	IYNREEIVRF	DSDVGEFRAV	TLLGLP**A**AEY	WNSQKDILER	KRAAVDRVCR	HNYQLELRTT	LQRR
17	7	w2	**0201*	positive	----------	----------	----------	----------	----------	----------	----------	----------	----------	----
1	1	w5	**0501*	positive	----------	---L------	-----G-T-H	------Y---	-----VY---	-PQ-R-**V**---	-----EV--G	A--S------	---EVAY-GI	----
2	w15	w6	**0602*	negative	--------F-	----------	-------T-Y	------YA--	-----VY---	-PQ-R-**D**---	-----EV--G	T--EL-T---	---EVAF-GI	----
		w7	**0301*	neutral-neg.†	----------	--A-------	-----Y-T-Y	------YA--	----EVY---	-PL-P-**D**---	-----EV---	T--EL-T---	----------	----
8	w8	w4	**0402*	neutral-neg.†	--------F-	----------	-----G-T-Y	------YA--	-----VY---	-P--RL**D**---	---------E	D--S--T---	----------	----
18	w13	w6	**0603*	neutral-neg.†			-----T-H	------YA--	-----VY---	-PQ-R-**D**---	-----EV--G	T--EL-T---		

Numbers above the sequences refer to the amino-acid position. The protein sequences are given in standard amino-acid single-letter code and aligned to the DQβ allele of the DRw17,DQw2 haplotype. A dash indicates identity with this sequence. Position 57, bold. The *DQB1* sequences are from the following sources: **0201* from refs 18, 19; **0501* from ref. 20; **0602* from ref. 26; **0301*, **0402* and **0603* from refs 1, 2.

† $P<0.05$ only before correction for multiple comparisons.

DQB1 alleles with negative and neutral-negative associations with IgA-D all have alanine at position 38 and threonine at position 77 (Table 2). As the *DQB1* alleles **0302* and **0604* with neutral to positive associations share these residues[1,2], we consider it is less likely that these positions are primarily involved in the non-susceptibility to IgA-D.

Position 57 of the HLA-DQβ chain has been shown to be the most informative genetic marker for insulin-dependent diabetes mellitus (IDDM) in Caucasians and blacks so far identified[1,2,12]. In IDDM Asp 57 confers resistance to the disease, whereas in the DRw6-associated subgroup of pemphigus vulgaris patients, Asp 57 is associated with disease susceptibility[3]. In a recent large population study of Norwegian IDDM patients[13] roughly the same frequencies of non-Asp 57 (82%) and Asp 57 homozygous patients (2%) were found as in the present study of IgA-D patients. This is intriguing, as the associations with serologically defined HLA class II specificities partly differ in the two diseases. A 10-fold increase in the prevalence of IgA-D has been described in IDDM[14]. Furthermore, a higher incidence of IDDM in IgA-D has been observed in a small series of patients[4]. The increased co-occurrence of the two diseases might possibly be attributed to the shared HLA-DQβ 57 association.

In IgA-D, as in IDDM, the HLA class II association cannot be explained merely by the charge of the amino acid at position 57 (refs 1, 2, 15–17). It is tempting to speculate, that the class II association(s) might be influenced by other gene products of the HLA region acting as minor histocompatibility antigens. In IgA-D the primary association in the DRw17,DQw2 and DR7,DQw2 haplotypes might be with the HLA-DQβ chain, as this is identical in the two haplotypes[18,19]. However, the sequences of the *DQB1* gene in DR1,DQw5 (positively associated with IgA-D) and DRw10,DQw5 (with a neutral association) are identical[20,21]. Thus, the *DQB1* allele of the DR1,DQw5 haplotype most probably interacts with an allele of another locus, which may be *DQA1* or *DRB1*. Furthermore, the protection conferred by the DRw15,DQw6 haplotype is more pronounced than the non-susceptibility of other Asp 57 positive DQβ alleles. Notwithstanding these exceptions, the data indicating that residue 57 might be important in conferring susceptibility and resistance to IgA-D are striking. These findings favour the hypothesis that the HLA class II molecules themselves might be involved in the pathogenesis of IgA-D. ☐

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Sequence homology shared by neurofibromatosis type-1 gene and *IRA-1* and *IRA-2* negative regulators of the *RAS* cyclic AMP pathway

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NEUROFIBROMATOSIS type-1 (NF-1) is one of the most frequently inherited genetic disorders affecting humans[1]. NF-1 primarily affects cells of neural crest origin and is characterized by patches of skin pigmentation (*café-au-lait* spots) and neurofibromas[2]. Cloning of the human *NF-1* gene shows that it encodes an 11–13 kilobase transcript that is frequently disrupted in NF-1 patients[3–5]. The frequent disruption of the *NF-1* gene in NF-1 patients combined with the autosomal dominant mode of inheritance of NF-1 strongly suggest that the *NF-1* gene is a tumour-suppressor gene. We have now sequenced a portion of the murine *NF-1* gene and show that the predicted amino-acid sequence is nearly the same as the corresponding region of the human *NF-1* gene product. Northern blotting identified mouse *NF-1* transcripts that are equivalent in size and complexity to those in human tissues, and Southern blotting shows that this region of the *NF-1* gene is evolutionarily well conserved. Finally, computer searches identified homology between the mouse *NF-1* gene and *IRA-1* and *IRA-2*, two genes identified in *Saccharomyces cerevisiae* that negatively regulate the *RAS*-cyclic AMP pathway. These findings provide important new insights into the possible function of the *NF-1* gene.

While cloning the human *NF-1* gene, we previously identified a complementary DNA clone, pmDV1, for the mouse *NF-1* gene. This clone contained a ~1.9-kilobase (kb) cDNA that we then used to identify cDNA clones for the human *NF-1* gene[3]. We determined the sequence of this 1.9-kb mouse cDNA (Fig. 1). The DNA sequence identifies a long open reading frame of 1,858 nucleotides. This sequence is completely contained in the sequence of the human *NF-1* gene[4]. Comparison of the mouse and human nucleotide sequences indicates that the two sequences are 91% identical. Among the 619 amino acids that can be compared between the two species, only three conservative amino-acid substitutions were observed (99.2% identity) (Fig. 1). These results indicate that this region of the mouse and human genes is highly conserved.

A 1.66-kb mouse NF1 probe generated by the polymerase chain reaction (PCR) (see Fig. 2 legend) was used in northern blots to identify transcripts from the murine *NF-1* locus (Fig. 2). In 4-day-old mice, an intense band of hybridization was detected in head RNA, faint hybridization detected in skin RNA, and no hybridization detected in body RNA (Fig. 2, lanes c–a, respectively). The size of this transcript is within the range of 11–13-kb reported for human *NF-1* transcripts.

In 14-day-old animals, *NF-1* transcripts were present in all tissues examined (Fig. 2, lanes d–g). Ubiquitous NF-1 expression also occurs in human tissues[5]. *NF-1* transcripts were also observed in the mouse B16 melanoma cell line (Fig. 2, lane h). In addition to the 11–13-kb transcript, two other transcripts were observed in melanoma RNA that were not detected in other tissues. It is not so far known whether these additional transcripts represent differential RNA splicing, different usage of 5′ or 3′ ends, or transcripts from a related gene. In summary, the complexity and size of *NF-1* transcripts identified in mice were similar to those observed in humans.

The evolutionary conservation of the *NF-1* gene was further explored by hybridizing the 1.66-kb mouse PCR *NF-1* probe to genomic DNA, obtained from a number of divergent species, that had been digested with restriction endonuclease (Fig. 3). At both moderate stringency (Fig. 3, top panel) and high stringency (Fig. 3, bottom panel) washes, bands of hybridization were readily detected in DNAs of all species, including species as evolutionarily distant as *Xenopus laevis* (Fig. 3, lane i). These results provide further evidence that the *NF-1* gene has been highly conserved during evolution and indicates that this region of the *NF-1* gene is functionally important.

For insight into the function of the *NF-1* gene, we tried to identify genes that share amino-acid sequence homology with the mouse *NF-1* gene product by searching protein computer databases (Fig. 4 legend). Two search programs, FASTA and SEQFP, identified amino-acid sequence homology between NF-1 and the *S. cerevisiae IRA-1* gene product (inhibitory regulator of the *RAS*-cAMP pathway[6]). By using SEQFP, we show that three non-overlapping 100-amino-acid regions of NF-1 have sequence homology with IRA-1, indicating that the region of homology with IRA-1 extends over a large region of the NF-1 protein. The amino-acid sequence comparison of mouse NF-1 and IRA-1 is shown in Fig. 4. The two genes have 20.9% amino-acid identity (42.8% amino-acid homology).

The statistical significance of the amino-acid homology was examined using the program RDF[7]. RDF determines the significance of similarity, z, between two sequences (see Fig. 4 legend). For $z > 6$ (initial or optimal score), the homology between the two sequences is probably significant, and for $z > 10$ (initial or optimal score), the homology is considered to be significant. We obtained an initial z score of 7.5 and an optimal z score of 19.2 for NF-1 and IRA-1, indicating that the amino-acid sequence homology between the two gene products is highly significant.

The sequence of an *IRA-1*-related gene, *IRA-2*, has been published[8]. *IRA-1* and *IRA-2* have 45% amino-acid identity. The amino-acid sequence of IRA-2 has not so far been incorporated into any of the genetic databases. Amino-acid sequence comparisons of IRA-2 and mouse NF-1 again identified sequence homology between the two gene products (data not shown). IRA-2 and NF-1 have 20.3% amino-acid identity (41.5% amino-acid similarity). The initial z score for NF-1 and IRA-2 proteins was 13.9 and the optimal z score was 17.5, indicating that the amino-acid sequence homology between these two genes is also highly significant.

Like the *NF-1* gene, *IRA-1* and *IRA-2* genes enclode large proteins[6–8] of 2,938 amino acids and 3,079 amino acids, respectively. Both genes are transcribed as 10-kb messenger RNAs. Analysis of disruption mutants of *IRA-1* and *IRA-2* indicates that both genes act additively to negatively regulate the *RAS*-cAMP pathway[8]. In *S. cerevisiae*, disruption mutants of either *IRA-1* or *IRA-2* are viable but result in increased sensitivity to heat-shock and nitrogen starvation, and are defective in sporulation.

Ras proteins are involved in the control of proliferation and differentiation in mammalian cells[9–10]. The activity of Ras proteins is modulated by their ability to bind and hydrolyse guanine nucleotides. GTP-binding activates Ras whereas GTP hydrolysis inactivates Ras. Mutant forms of Ras found in human tumours have greatly decreased GTPase activity, resulting in accumulation of Ras in the GTP-bound active form. Ras activity is normally regulated by accessory proteins that alter its ability to bind and hydrolyse GTP. For example, mammalian cells possess GTPase-activating proteins (GAP proteins) that catalytically accelerate hydrolysis of GTP bound to Ras protein[11].

In *S. cerevisiae*, RAS proteins control the activity of adenylyl cyclase that produces the second messenger, cAMP. RAS

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   1 TAT ATG ACC CCA TGG CTG TCA AAT CTA GTC CGT TTT TGT AAG CAT AAT    48
   1 Y   M   T   P   W   L   S   N   L   V   R   F   C   K   H   N      16

  49 GAT GAT GCC AAA CGA CAA AGG GTT ACT GCC ATC CTT GAT AAG CTA ATA    96
  17 D   D   A   K   R   Q   R   V   T   A   I   L   D   K   L   I      32

  97 ACA ATG ACT ATA AAC GAG AAG CAG ATG TAT CCT TCT ATT CAA GCA AAA   144
  33 T   M   T   I   N   E   K   Q   M   Y   P   S   I   Q   A   K      48

 145 ATC TGG GGG AGC CTT GGG CAG ATC ACA GAC CTG CTG GAT GTT GTG CTT   192
  49 I   W   G   S   L   G   Q   I   T   D   L   L   D   V   V   L      64

 193 GAC AGT TTC ATC AAG ACC AGT GCG ACA GGA GGC TTA GGG TCT ATC AAA   240
  65 D   S   F   I   K   T   S   A   T   G   G   L   G   S   I   K      80

 241 GCT GAG GTG ATG GCA GAC ACA GCT GTG GCT TTA GCT TCT GGA AAT GTG   288
  81 A   E   V   M   A   D   T   A   V   A   L   A   S   G   N   V      96

 289 AAA TTG GTG TCG AGT AAG GTT ATT GGA AGG ATG TGT AAA ATA ATT GAC   336
  97 K   L   V   S   S   K   V   I   G   R   M   C   K   I   I   D     112

 337 AAG ACT TGC TTA TCC CCA ACT CCA ACT TTA GAA CAG CAT CTT ATG TGG   384
 113 K   T   C   L   S   P   T   P   T   L   E   Q   H   L   M   W     128

 385 GAC GAC ATT GCC ATT TTA GCC CGC TAC ATG CTG ATG CTG TCC TTC AAC   432
 129 D   D   I   A   I   L   A   R   Y   M   L   M   L   S   F   N     144

 433 AAC TCC CTC GAT GTG GCG GCT CAT CTG CCC TAT CTC TTC CAT GTT GTC   480
 145 N   S   L   D   V   A   A   H   L   P   Y   L   F   H   V   V     160

 481 ACT TTC TTA GTA GCC ACA GGT CCC TTG TCC CTC CGA GCT TCC ACA CAT   528
 161 T   F   L   V   A   T   G   P   L   S   L   R   A   S   T   H     176

 529 GGG CTG CTC ATC AAT ATC ATT CAC TCT CTG TGT ACT TGT TCT CAG CTT   576
 177 G   L   L   I   N   I   I   H   S   L   C   T   C   S   Q   L     192
             V
 577 CAC TTT AGT GAA GAG ACC AAG CAA GTT TTG AGG CTC AGT CTA ACA GAG   624
 193 H   F   S   E   E   T   K   Q   V   L   R   L   S   L   T   E     208

 625 TTC TCG TTA CCC AAA TTT TAC TTA CTG TTT GGC ATT AGC AAA GTC AAG   672
 209 F   S   L   P   K   F   Y   L   L   F   G   I   S   K   V   K     224

 673 TCG GCT GCT GTC ATT GCC TTC CGT TCC AGT TAC CGG GAC CGC TCC TTC   720
 225 S   A   A   V   I   A   F   R   S   S   Y   R   D   R   S   F     240

 721 TCC CCT GGC TCC TAT GAG AGG GAG ACT TTT GCT TTG ACG TCC CTG GAA   768
 241 S   P   G   S   Y   E   R   E   T   F   A   L   T   S   L   E     256

 769 ACA GTC ACA GAA GCT TTG TTG GAG ATC ATG GAG GCA TGT ATG AGA GAT   816
 257 T   V   T   E   A   L   L   E   I   M   E   A   C   M   R   D     272

 817 ATT CCA ACA TGC AAG TGG CTG GAT CAG TGG ACA GAA CTA GCT CAA AGA   864
 273 I   P   T   C   K   W   L   D   Q   W   T   E   L   A   Q   R     288

 865 TTT GCG TTT CAG TAT AAC CCA TCG CTG CAG CCA AGA GCT CTT GTG GTG   912
 289 F   A   F   Q   Y   N   P   S   L   Q   P   R   A   L   V   V     304

 913 TTT GGC TGT ATT AGC AAA CGA GTG TCT CAT GGG CAG ATA AAG CAG ATT   960
 305 F   G   C   I   S   K   R   V   S   H   G   Q   I   K   Q   I     320

 961 ATC CGA ATT CTT AGC AAG GCA CTT GAA AGT TGT TTA AAA GGA CCT GAC  1008
 321 I   R   I   L   S   K   A   L   E   S   C   L   K   G   P   D     336

1009 ACT TAC AAC AGT CAA GTT CTG ATA GAA TCT ACG GTG ATA GCA CTA ACA  1056
 337 T   Y   N   S   Q   V   L   I   E   S   T   V   I   A   L   T     352
                                         A
1057 AAA TTA CAG CCG CTT CTT AAT AAG GAC TCG CCC CTG CAC AAA GCC CTC  1104
 353 K   L   Q   P   L   L   N   K   D   S   P   L   H   K   A   L     368

1105 TTT TGG GTT GCT GTG GCT GTG CTG CAG CTG GAC GAA GTC AAC TTG TAT  1152
 369 F   W   V   A   V   A   V   L   Q   L   D   E   V   N   L   Y     384

1153 TCA GCC GGC ACT GCA CTT CTG GAA CAA AAC CTG CAC ACC TTG GAC AGT  1200
 385 S   A   G   T   A   L   L   E   Q   N   L   H   T   L   D   S     400

1201 CTC CGG ATA TTC AAT GAC AAG AGT CCG GAA GAA GTA TTT ATG GCG ATC  1248
 401 L   R   I   F   N   D   K   S   P   E   E   V   F   M   A   I     416

1249 CGG AAC CCT CTG GAA TGG CAC TGC AAG CAG ATG GAT CAC TTT GTC GGA  1296
 417 R   N   P   L   E   W   H   C   K   Q   M   D   H   F   V   G     432

1297 CTC AAC TTC AAC TCT AAC TTT AAC TTT GCA CTA GTT GGA CAC CTC TTA  1344
 433 L   N   F   N   S   N   F   N   F   A   L   V   G   H   L   L     448

1345 AAA GGG TAC AGG CAT CCT TCA CCT GCC ATT GTT GCA AGA ACA GTC AGA  1392
 449 K   G   Y   R   H   P   S   P   A   I   V   A   R   T   V   R     464

1393 ATT TTG CAT ACA CTA CTA ACT CTC GTT AAC AAA CAT AGA AAT TGT GAC  1440
 465 I   L   H   T   L   L   T   L   V   N   K   H   R   N   C   D     480

1441 AAA TTT GAG GTG AAT ACA CAG AGT GTG GCT TAC TTA GCA GCT CTA CTC  1488
 481 K   F   E   V   N   T   Q   S   V   A   Y   L   A   A   L   L     496

1489 ACA GTG TCT GAA GAG GTT CGA AGT CGC TGT AGC CTC AAG CAT AGG AAG  1536
 497 T   V   S   E   E   V   R   S   R   C   S   L   K   H   R   K     512

1537 TCC CTT CTT CTT ACT GAT ATT TCA ATG GAA AAT GTT CCT ATG GAT ACA  1584
 513 S   L   L   L   T   D   I   S   M   E   N   V   P   M   D   T     528

1585 TAT CCC ATT CAT CAC GGT GAC CCC AGC TAT AGG ACA CTA AAG GAG ACC  1632
 529 Y   P   I   H   H   G   D   P   S   Y   R   T   L   K   E   T     544

1633 CAG CCA TGG TCC TCT CCC AAA GGT TCA GAA GGA TAC CTT GCA GCC ACC  1680
 545 Q   P   W   S   S   P   K   G   S   E   G   Y   L   A   A   T     560

1681 TAC CCA GCT GTA GGC CAA ACC AGT CCC CGA GCC AGG AAA TCC ATG AGC  1728
 561 Y   P   A   V   G   Q   T   S   P   R   A   R   K   S   M   S     576
             T
1729 TTG GAT ATG GGA CAG CCT TCT CAG GCC AAC ACA AAA AAA TTG CTT GGA  1776
 577 L   D   M   G   Q   P   S   Q   A   N   T   K   K   L   L   G     592

1777 ACA AGA AAA AGT TTT GAT CAC TTG ATA TCT GAC ACA AAG GCT CCT AAA  1824
 593 T   R   K   S   F   D   H   L   I   S   D   T   K   A   P   K     608

1825 AGA CAA GAA ATG GAA TCA GGG ATT ACA ACA CCC C                    1858
 609 R   Q   E   M   E   S   G   I   T   T   P                         619
```

FIG. 1 Sequence of the murine *NF-1* cDNA clone. The 1,858 nucleotides of the pmDV1 cDNA clone that corresponds to the mouse *NF-1* gene are shown with the predicted product (single-letter amino-acid code). The numbers correspond to the nucleotide (upper line in each pair) or amino acid (lower line in each pair). The three amino-acid differences between the predicted murine amino-acid sequence and the predicted human amino-acid sequence are shown by the shaded boxes; the amino-acid differences occur at positions 179, 346 and 563.

METHODS. The mouse NF-1 cDNA clone (pmDV1) was isolated from a mouse macrophage cDNA library[3] and was sequenced by the dideoxy chain termination method[18] using a Sequenase kit (USB) and double-stranded plasmid DNA. Sequencing primers were either the T3 or T7 polymerase oligonucleotide primers or synthetic oligonucleotides from previously determined sequences. The 5′ end of the plasmid insert had a run of 72 T residues flanked on either end by *Eco*RI linkers; this sequence is presumably a cloning artefact, as it is not represented in the human cDNA clones[4].

proteins in *S. cerevisiae*, like those in mammalian cells, are regulated by several accessory proteins. The CDC25 protein positively regulates RAS function by catalysing the exchange of GDP bound to RAS for GTP[12-15]. By contrast, IRA-1 and IRA-2 proteins suppress the activity of RAS by GTP hydrolysis of RAS-GTP. The phenotype of yeast cells that have lost this negative regulatory function resembles that of yeast cells carrying the constitutively active form of RAS found in human tumours. Both IRA-1 and IRA-2 share limited but significant amino-acid sequence homology with mammalian GAP, and mammalian GAP can suppress the phenotype of *ira* mutants[16]. This suggests that IRA-1 and IRA-2 may be functionally equivalent to mammalian GAP.

The GAP homology domain of IRA-1 and IRA-2 has been localized to a small region in the middle of both proteins in amino acids 1,451–1,780 (IRA-1) and 1,597–1,925 (IRA-2)[16].

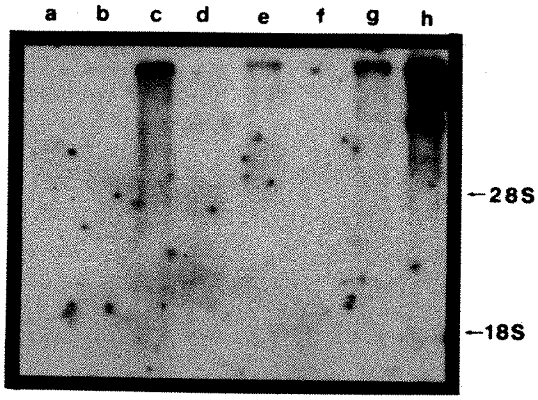

FIG. 2 Expression of murine *NF-1* mRNA. Poly(A)$^+$ RNA (twice selected) was examined by northern blotting using the mouse NF-1 PCR probe. The RNA (5 μg per lane) was from body (a), skin (b) or head (c) of 4-day-old DBA/2J mice, or spleen and thymus (d), kidney (e), liver (f), or brain (g) of 14-day-old DBA/2J mice, and B16 melanoma cell line (h). The position of migration of 28S and 18S RNA is shown. Less RNA was loaded in lanes a and b than in the other lanes, as determined by hybridization with a β-actin control probe (data not shown).

METHODS. Total RNA was isolated from tissues using the LiCl-urea method[19]. Poly(A)$^+$ twice-selected RNA[20] was size-fractionated on 1.0% agarose-formaldehyde gels[21]. The DNA probe used for both Southern and northern blot hybridization was PCR-generated using the mouse *NF-1* cDNA clone pmDV1[3] as a template. The amplimers (CATAATGATGATGCCAAACGA and GACTGGTTTGGCCTACAGCT) used to generate the probe were derived from the known *NF-1* DNA sequence and the resulting 1.66-kb probe was purified on an agarose gel and was devoid of plasmid sequences. Northern blots were hybridized and washed as described[22].

The small size of the essential GAP domain and the large size of the IRA proteins suggests that sequences flanking the GAP domain may have important regulatory functions. For example, IRA proteins seem to function as detectors of nutritional limitation. Therefore, one role for these flanking sequences may be to monitor the level of nutrients in the external environment and transduce that information to the GAP catalytic domain, which, in turn, effects the level of GTP bound to RAS.

The amino-acid sequence homology we have observed between the *NF-1* and *IRA* gene products, combined with the unusually large size of each transcript, raises the possibility that the *NF-1* and *IRA* genes have a common ancestor and that *NF-1* functions as a negative regulator of signal transduction in mammalian cells in a manner analogous to the *IRA* genes in *S. cerevisiae.* Such a mode of action of the *NF-1* gene would explain the basis for the uncontrolled growth of neural crest-derived cells seen in patients with mutations in the *NF-1* gene and would also be consistent with a tumour-suppressor gene, which is the postulated mode of action of the *NF-1* gene. Additional sequencing studies of the *NF-1* gene coupled with the development of biological assays for the *NF-1* gene will be necessary to confirm this possibility. The homology we have detected between NF-1 and IRA is in the C terminus of each protein[4,5]. As additional segments of the *NF-1* gene are cloned and sequenced, it will be interesting to see if the homology we have detected between NF-1 and IRA extends into the N-terminal and GAP domains of the IRA proteins.

The strong evolutionary conservation of the *NF-1* gene suggests that model organisms such as the mouse will be useful for developing assays for elucidating the functions of the *NF-1* gene. For example, mutations can now be introduced into the mouse *NF-1* gene using techniques for homologous recombination in mouse embryonic stem cells. Not only will this approach shed further light into the functions of the *NF-1* gene, but it may also produce a useful mouse model for the study NF-1 disease.

The identification of two related *IRA* genes in yeast raises the possibility that there are many *IRA*-related genes in

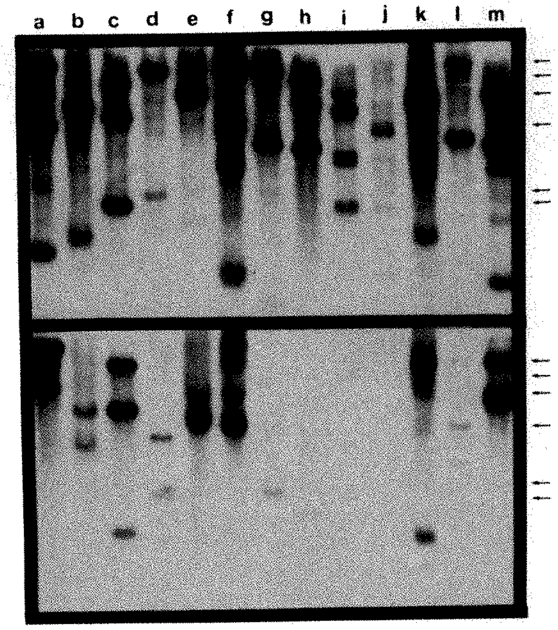

FIG. 3 The *NF-1* gene has been well-conserved throughout evolution. Genomic DNAs from a variety of different species were cleaved with either *Eco*RI (upper panel) or *Hin*dIII (lower panel) and examined for sequences that crosshybridize to the mouse *NF-1* PCR probe on Southern blotting. The hybridization was performed in 4 × SSCP at 55 °C for 16 h with a final wash in either 1 × SSCP 55 °C (upper panel) or 0.2 × SSCP 65 °C (lower panel). The DNA (10 μg per lane) was isolated from hamster (a), guinea-pig (b), rat (c), mink (d), dog (e), cat (f), chicken (g), turkey (h), *Xenopus laevis* (i), rabbit (j), sheep (k), human (l) or C57BL/6J mice (m). The arrows indicate the mobility of *Hin*dIII-cleaved lambda DNA electrophoresed in parallel lanes of the same gels and correspond to 23.1, 9.4, 6.6, 4.4, 2.3 and 2.0 kb (from top to bottom).

METHODS. High-relative molecular mass DNA was prepared from frozen tissue samples as described[23]. Restriction endonuclease digestions, agarose gel electrophoresis and Southern blot transfers were performed as described[24]. Hybridization was at 55 °C in 4 × SSCP, 0.1% SDS, 10% dextran sulphate, 4 × Denhardt's solution and 0.1 mg ml^{-1} salmon sperm DNA. Washes were as follows: three times in 1 × SSCP, 0.1% SDS at 55 °C, for 30 min each (*Eco*RI Southern blot); and for the *Hin*dIII Southern blot, additional washes of 0.5 × SSCP, 0.1% SDS at 65 °C and 0.2 × SSCP, 0.1% SDS at 65 °C were performed for 30 min each.

```
   1 ......YMTPWLSNLV.RFCKHNDDAKRQRVTAILDKLITMTINEKQMYP   43
            :: |::||  : :  ||:   : :: |:  || :|: |  : :
2270 IPTVVCSLSYWVPNLYEHVYLANDEEGPEAISRIIYSLIRLTVKEPNFTT 2319

  44 SIQAKIWGSLGQITDLLDVVLDSFIKTSATGGLGSIKAEVMADTAVALAS   93
     :    ||   |: : | :|::: ::  | : : :|     :|  :: |:|
2320 AYLQQIWFLLALDGRLTNVIVEEIV..SHALDRDSENRDWMKAVSI.LTS 2366

  94 GNVKLVSSKVIGRMCKIIDKTCLSPTPTLEQHLMWDDIAILARYMLMLSF  143
          :::  ||::  :| |: |:: :       | :::||::  : : |
2367 FPTTEIACQVIEKLINMI.KSFLPSLAVEASAHSWSELTILSKISVSIFF 2415

 144 NNSLDVAAHLPYLFHVVTFLVATGPLSLRASTHGLLINIIHSLCTCSQLH  193
     : :|      || :: |::|:  ||   :| |  :||:|: |||     |
2416 ESPLLSQMYLPEILFAVSLLIDVGPSEIRVSLYELLMNVCHSLTNNESL. 2464

 194 FSEETKQVLRLSLTEFSLPKFYLLFGISKVKSAAVIAFRSSYRDRSFSPG  243
      :|  :  | :  : |:  |: :: |:|  |:  :  | :|    |||:
2465 .PERNRKNLDIVCATFARQKLNFISGFSQEKGRVLPNFAAS....SFSS. 2508

 244 SYERETFALTSLETVTEALLEIMEACMRDIPTCKWLDQWTELAQRFAFQY  293
             :::|:  |  :: :|| :        |   :        |
2509 .......KFGTLDLFTKNIMLLME.YGSISEGAQWEAKYKKYLMDAIFGH 2550

 294 NPSLQPRALVVFGCISKRVSHGQIKQIIRILSKALESCLKGPDTYNSQVL  343
      : : :||::::| :||  ||: :       :| ::    :    :   :
2551 RSFFSARAMMILGIMSK..SHTSLFLCKELLVETMKVFAEPVVDDEQMFI 2598

 344 IESTVIALTKLQPLLNKDSPLHKALFWVAVAVLQLDEVNLYSAGTALLEQ  393
     | : |:: :|:   |:  | | | |||:|   ::     |: :|  ::
2599 IIAHVFTYSKIVEGLDPSSELMKELFWLATICVESPHPLLFEGGLLFMVN 2648

 394 NLHTLDSLRI...FNDKSPEEVFMAIRNPLEWHCKQMDHFVGLNFN.SNF  439
      |  | ::::   |::||     :|  ||       :: : |  :|  ||
2649 CLKRLYTVHLQLGFDGKSLAKKLMESRNFAATLLAKLESYNGCIWNEDNF 2698

 440 NFALVGHLLKGYRHPSPAIVARTVRILHTLLT..................  471
        ::| :  |   : | : : ::   |::|:
2699 PHIILGFIANGL..SIPVVKGAALDCLQALFKNTYYERKSNPKSSDYLCY 2746

 472 ..LVNKHRNCDKFEVNTQSVAYLAALLTVSEEVRSRCSLKHRKS......  513
       |::     : :      |::    |::: : ::   :| :: |
2747 LFLLHLVLSPEQLSTLLLEVGFEDELVPLNNTLKVPLTLINWLSSDSDKS 2796

 514 .......LLLTDISMENVP............MDTYPIHHGDPSYRTLKET  544
             ||    | : |            :   ||       | ||
2797 NIVLYQGALLFSCVMSDEPCKFRFALLMRYLLKVNPICVFRFYTLTRKEF 2846

 545 QPWSSPKGSEGYLAATYPAVGQTSPRARKSMSLDM.GQPSQANTKKLLGT  593
       :|:   | : :| ::  :|   :::      :: ::  :   |: |:
2847 RRLSTLEQSSEAVAVSFELIGMLVTHSEFNYLEEFNDEMVELLKKRGLSV 2896

 594 RKSFDHLISDTKAPKRQEMESGITTPRN..............          621
      |::| :  :     : | :  ::
2897 VKPLDIFDQEHIEKLKGEGEHQVAIYERKRLATMILARMSCS          2938
```

FIG. 4 Amino-acid (single-letter code) sequence comparison of mouse NF-1 and yeast IRA-1. Alignment of the known predicted amino-acid sequence of mouse NF-1 (top sequence) with the C-terminal region of IRA-1 (lower sequence). Identical amino acids are shown by the solid vertical line, conservative amino-acid substitutions are shown by the colons (these amino acids are grouped as ILMV, KHR, DENQ, STPAG and FWY; single-letter code). Alignment was performed using the GAP program in the University of Wisconsin Genetics Computer Group package[25]. The periods indicate the placement of gaps to maximize alignment between the sequences.

METHODS. Sequences were analysed using the software package of the Genetics Computer Group[25]. Homology to IRA-1 and IRA-2 was identified using both the FASTA program[26] and the SEQFP program in the IDEAS package[27]. The Genbank (release 64.0), NBRF (release 25.0), EMBL (release 22.0) and SwissProt (release 14.0) databases were screened for homologous sequences. SEQFP and FASTA are based on the same algorithm[7]. Alignment scores (z values) were determined using the program RDF[7]. RDF determines both the initial (without introduction of gaps) and optimal (after allowing for insertions and deletions) scores of a sequence alignment and then randomizes one of the sequences many times (100) and determines the mean of the random scores. The z values were determined using ktup = 2 and $N = 100$.

mammals. Indeed, a second form of neurofibromatosis has been identified, neurofibromatosis type-2 (NF-2). Mapping studies indicate that the *NF-2* locus, in at least some families, is located on human chromosome 22 (ref. 17). Once the *NF-2* gene is cloned it will be important to determine whether it too shares sequence homology with *IRA*-related genes. ☐

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Frequent activation of N-*myc* genes by hepadnavirus insertion in woodchuck liver tumours

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THE recent finding of c-*myc* activation by insertion of woodchuck hepatitis virus DNA in two independent hepatocellular carcinoma[1] has given support to the hypothesis that integration of hepatitis B viruses into the host genome, observed in most human and woodchuck liver tumours[2,3], might contribute to oncogenesis. We report here high frequency of woodchuck hepatitis virus DNA integrations in two newly identified N-*myc* genes: N-*myc1*, the homologue of known mammalian N-*myc* genes, and N-*myc2*, an intronless 'complementary DNA gene' or 'retroposon' that has retained extensive coding and transforming homology with N-*myc*. N-*myc2* is totally silent in normal liver, but is overexpressed without genetic rearrangements in most liver tumours. Moreover, viral integrations occur within either N-*myc1* or N-*myc2* in about 20% of the tumours, giving rise to chimaeric messenger RNAs in which the 3′ untranslated region of N-*myc* was replaced by woodchuck hepatitis virus sequences encompassing the viral enhancer. Insertion sites were clustered in a short sequence of the third exon that coincides with a retroviral integration hotspot within the murine N-*myc* gene, recently described in T-cell lymphomas induced by murine leukaemia virus[4-6]. Thus, comparable mechanisms, leading to deregulated expression of N-*myc* genes, may operate in the development of tumours induced either by hepatitis virus or by nonacute retroviruses in rodents. Activation of *myc* genes by insertion of hepadnavirus DNA now emerges as a common event in the genesis of woodchuck hepatocellular carcinoma.

We have cloned and sequenced a single woodchuck hepatitis virus (WHV) insertion site from an early hepatocellular carcinoma (HCC) carried by a chronically infected woodchuck (W204). The WHV insert, a subgenomic fragment encompassing the viral enhancer, is flanked by host sequences that are not contiguous in the normal woodchuck genome (Fig. 1*c*). We subcloned a 1.7-kilobase (kb) DNA fragment of the 5′ host-viral junction (BA1700, Fig. 1*c*), showing homology with N-*myc* sequences and no repetitive elements, and used this to further screen the W204 HCC genomic library. This approach yielded two subsets of clones, derived from distinct genomic loci. The locus depicted in Fig. 1*a* was identified by nucleotide sequencing as the woodchuck homologue of the human and murine N-*myc* genes[7-9], similarly organized into three exons. It was thereafter designated as N-*myc1*. The second locus, termed N-*myc2*, represents the unoccupied allele of the 5′ flanking cellular sequences in W204 HCC (Fig. 1*b*), and is part of the normal woodchuck genome, as shown by Southern analysis (Fig. 3).

Nucleotide sequencing of N-*myc2* (Fig. 2*a*) revealed a structure typical of a cDNA gene or retroposon. The N-*myc* homology starts at the exact 5′ end of the first exon[8], extends through exon 1 to the first splice donor site[10], then over the entire exons 2 and 3, showing no intervening sequences. It terminates at the 3′ end of the third exon, 16 base pairs (bp) downstream of a consensus polyadenylation sequence, and is flanked at its 3′ side by an adenosine-rich stretch of 53 nucleotides, which might constitute the remnants of a $poly(A)^+$ tail. A sequence of 16 nucleotides is directly repeated at each end of the N-*myc*-like sequence (positions −325 and 2,241, Fig. 2*a*). These data indicate that N-*myc2* may have evolved from retroposition of one of the two alternatively spliced N-*myc* mRNAs[10]. This process, or the subsequent absence of selective pressure, is usually associated with genetic lesions that prevent translation of any transcript into functional polypeptides[11]. However, a N-*myc*-like open reading frame is preserved in the normal N-*myc2* allele (Fig. 2), as well as in the mutated allele of W204 HCC, interrupted by WHV DNA insertion 6 bp downstream of the stop codon (Fig. 1*c*).

The predicted N-*myc2* coding region encodes a polypeptide of 454 amino acids showing 80% and 78% homology with the woodchuck and human N-Myc proteins and extensive conservation of the functional domains of known Myc proteins (Fig. 2*b*). As in other N-*myc* genes, the coding region begins with three ATG codons, closely spaced and in-phase; the first and the second ATG triplets of the human N-*myc* gene encode the N-terminal methionine residues of two N-*myc* polypeptides[12]. The stretch of acidic amino acids around position 270 of N-Myc2, common to c-Myc and N-Myc proteins, possibly coincides with a transcriptional activation region[13] and/or with a protein instability motif[14]. The highest degree of homology between N-Myc2 and other N-Myc proteins is encountered in the carboxy terminal region, which contains a basic motif and an amphipathic helix-loop-helix bearing a leucine zipper, as indicated in Fig. 2*b*. This complex structure, common to Myc proteins and other regulatory factors, is believed to be critical for specific DNA-binding and protein dimerization functions[15,16] and is important for the transforming activity of c-Myc[17] and N-Myc[18].

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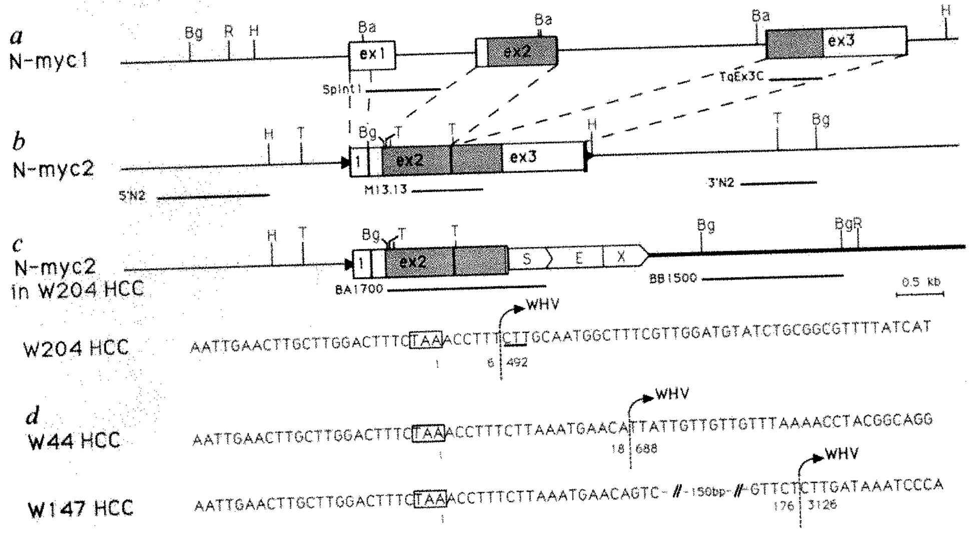
A characteristic functional property of mammalian *myc* genes is their ability to cooperate with an activated *ras* gene in tumorigenic conversion of primary embryo fibroblasts[19-21]. To assess whether N-*myc2* has retained this function, we cotransfected pRSV-N-Myc2 (a plasmid carrying the 2,627-bp *Rsa*I-*Hin*dIII fragment of N-*myc2* cloned into the *Hin*dIII and *Hpa*I sites of pRSVCAT) and pSV$_2$neo-ras (a c-Ha-*ras1* (EJ)[22] expression vector) into rat embryo fibroblasts using standard conditions[19]. Neomycin-resistent foci of transformed cells were obtained with the same efficiency as in rat embryo fibroblasts transfected with pSV-c-myc-1[23] and pSV$_2$neo-ras. When foci carrying EJras together with either N-*myc2* or c-*myc* genes were picked, they yielded established lines of overtly transformed, rapidly growing cells (manuscript in preparation). Both types of cotransfected cells were consistently tumorigenic when injected into nude mice, producing tumours that reached diameters of 1-3 cm, two weeks after inoculation (results not shown). Thus, N-*myc2* can replace c-*myc* in rat embryo fibroblasts cotransformation and tumorigenicity assays.

A survey of 30 fresh, independent liver tumours, using Southern blot hybridization of *Hin*dIII-digested genomic DNA with a N-*myc* probe revealed DNA rearrangements of a N-*myc* allele in six additional tumours (Fig. 3*a*). In all cases except W97 HCC, the tumour-specific N-*myc* bands comigrated with viral bands (Fig. 3*b*), suggesting junction fragments of N-*myc* and integrated viral sequences as in W204 HCC. Remarkably, all rearranged *Hin*dIII fragments of individual tumours annealed to probe WHV En, a 600-bp subgenomic fragment encompassing the viral enhancer element[24] (Fig. 3*b*). Only in W134B HCC could the tumour-specific band also be revealed by a N-*myc1*-specific probe (Fig. 3*c*). Rearrangements of N-*myc2* were confirmed in the remaining six tumours, using *Bam*HI digests and the N-*myc2*-specific probes illustrated in Fig. 1 (data not shown). In addition, *Bam*HI or *Taq*I digestion of tumour DNAs and hybridization with a probe of the N-*myc1* exon-3 coding region localized the integration sites in the third exon of N-*myc1* (in W134TB) or in the homologous region of N-*myc2* (in the remaining tumours) (Fig. 3*d*).

Elevated levels of N-*myc*-related transcripts ranging from 3-4.5 kb in size were detected in four woodchuck HCCs (W147, 44, 134B and 264) (Fig. 4*a*). Fainter bands at 3.4 and 4.2 kb in W204 HCC and at 3.5 kb in 1337 HCC were seen after longer exposures. Northern blot hybridization with probe WHV En identified viral transcripts of abnormal sizes, comigrating with N-*myc*-related transcripts in all six tumours (Fig. 4*b*), suggesting co-transcription of N-*myc* and viral sequences from the mutated alleles. This was confirmed in W44 and W147 HCCs by a polymerase chain reaction (PCR) procedure on cDNA made from total cellular RNA, using oligonucleotides located on each side of the putative host-viral junctions and sequencing of the amplification products (Fig. 1*d*). In both tumours, as in W204 HCC, integration of viral DNA directly downstream to the N-*myc2* coding region and in the same transcriptional orientation produced truncated N-*myc* RNAs in which the 3' untranslated region of N-*myc* was replaced by WHV sequences. The viral enhancer was retained in these transcripts, as shown by northern hybridization (Fig. 4*b*) and sequencing of cloned DNA fragments from the host-viral junctions (Fig. 1*c*, *d*). Furthermore, the host-viral junctions in the three tumours analysed are localized in different parts of the WHV pre-S/S region, but less than 200 bp apart within the third N-*myc2* exon. These sequences, previously identified as the unique target for retroviral insertion into the murine N-*myc* gene in murine leukaemia virus-induced T cell lymphomas[4-6], might be highly recombinogenic for reasons not yet understood.

Several other woodchuck tumours produce abundant 2.3-kb N-*myc* transcripts in the absence of gross genetic alterations of the N-*myc* genes (Fig. 4*a*). These RNAs were not detected by a N-*myc1*-specific probe (Fig. 4*c*) and were identified as N-*myc2* transcripts by PCR experiments using cDNA made from total


a
N-myc1
Bg
R
H
Ba
ex1
Ba
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Ba
ex3
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SpInt1
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b
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H
T
Bg
T
T
H
1
ex2
ex3
T
Bg
5'N2
M13.13
3'N2
c
N-myc2
in W204 HCC
H
T
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1
ex2
S
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X
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BgR
BA1700
BB1500
0.5 kb
WHV
W204 HCC
AATTGAACTTGCTTGGACTTTCTAAACCTTTCTTGCAATGGCTTTCGTTGGATGTATCTGCGGCGTTTTATCAT
1
6
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d
WHV
W44 HCC
AATTGAACTTGCTTGGACTTTCTAAACCTTTCTTAAATGAACATTATTGTTGTTGTTTAAAACCTACGGCAGG
1
18
688
WHV
W147 HCC
AATTGAACTTGCTTGGACTTTCTAAACCTTTCTTAAATGAACAGTC-//-150bp-//-GTTCTCTTGATAAATCCCA
1
176
3126

FIG. 1 Structural comparison of N-*myc1*, N-*myc2*, and rearranged N-*myc2* alleles. *a*, Organization of N-*myc1*. Boxes, the exons with coding region shaded. SpInt1 is a N-*myc1*-specific probe, TqEx3C detects both N-*myc* genes. Ba, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; *Eco*RI; T, *Taq*I (not all sites are shown). *b*, Organization of the germline N-*myc2* allele. Regions of homology with N-*myc1* exons are aligned with dotted lines. The 3' poly(A) stretch is represented as a thick vertical bar and the flanking direct repeats as two black arrowheads. 5'N2 and 3'N2 are N-*myc2*-specific probes, M13.13 hybridizes with N-*myc1* and N-*myc2*. *c*, Restriction map of the occupied N-*myc2* allele in W204 HCC and sequence of the left host-virus DNA junction. Narrow open box, the 1.5 kb WHV insert indicating the viral (+) strand orientation. S and X, viral genes; E, enhancer element. Thin line, N-*myc2* locus; thick line, 3' flanking cellular sequences different from N-*myc2*. *N-myc2* sequences are numbered relative to the last base pair of the stop codon (boxed) and WHV sequences from ref. 34. The host-viral junction is indicated by an arrow and the underlined triplet CTT corresponds to junctional homology of viral and cellular DNA. *d*, Sequence analysis of the N-*myc2*-WHV junction in W44 HCC and W147 HCC chimaeric transcripts.

METHODS. An Embl3 recombinant phage library was constructed from size-fractionated DNA of W204 HCC partially digested by *Sau*3A and screened with a probe for WHV DNA[1]. Restriction fragments from the viral-host junctions (BA1700, a 1.7-kb *Bgl*II-*Apa*I fragment and BB1500, a 1.5-kb *Bgl*II fragment) were subcloned into Bluescript vectors (Stratagene). The N-*myc1* and N-*myc2* genomic clones were obtained by screening the same library with BA1700 as a probe. Restriction fragments from the N-*myc1* gene (SpIntI, a 800-bp *Sal*I-*Sph*I fragment, and TqEx3C, a 450 bp *Taq*I fragment) and from the germline N-*myc2* allele (5'N2, a 1.2-kb *Sal*I-*Hin*dIII fragment and 3'N2, a 800-bp *Xba*I-*Bgl*II fragment) were cloned and used as probes. The nucleotide sequence of the N-*myc1* gene will be published elsewhere. The 3.5-kb *Hin*dIII fragment of the germline N-*myc2* allele and the 6.4-kb *Hin*dIII-*Eco*RI fragment of the viral insertion site were fully sequenced[35] using a shotgun strategy[36]. M13.13, a 750-bp insert from a M13 clone, was subsequently subcloned into Bluescript. For analysis of the chimaeric transcripts from W44 and W147 HCCs, the reverse transcriptase-polymerase chain reactions were performed essentially as described[37], using as primers a WHV-specific oligonucleotide of the viral enhancer core (position 1,309-1,328) and the N-*myc2*-specific oligonucleotide OLNM3 (see Fig. 2). Omission of the reverse transcriptase or testing normal liver RNA yielded parallel negative controls.

cellular RNA and N-*myc2*-specific primers (see Fig. 2) (data not shown). We observed varying levels of the 2.3-kb N-*myc2* RNA in 18 out of 30 woodchuck HCCs, but no detectable expression of N-*myc2* in the non-tumorous parts of the same livers or in the livers of WHV-infected or uninfected animals (Fig. 4*a*). Thus, N-*myc2* expression seems to be modulated not only in response to genetic damage, but also by *trans*-acting factors produced in a majority of woodchuck HCCs. The reduced size of N-*myc2* transcripts as compared with the 2.9-kb human N-*myc* mRNA (Fig. 4*a*, lane 9) suggests that transcriptional initiation and/or termination sites are located within the retroposed sequences, as in two documented cases of expressed retroposons[25,26]. Indeed, evidence for alternatively spliced N-*myc* mRNA, using two different 5′ leader sequences in the first exon, indicates that part of the normal N-*myc* promoter might reside within the 5′ region of the first exon (exon 1a)[10], which is retained in N-*myc2* (see Fig. 1 and 2).

In conclusion, the analysis of a viral insertion site in a woodchuck HCC has led to the identification of a novel intronless N-*myc* gene that retains transforming potential and is frequently

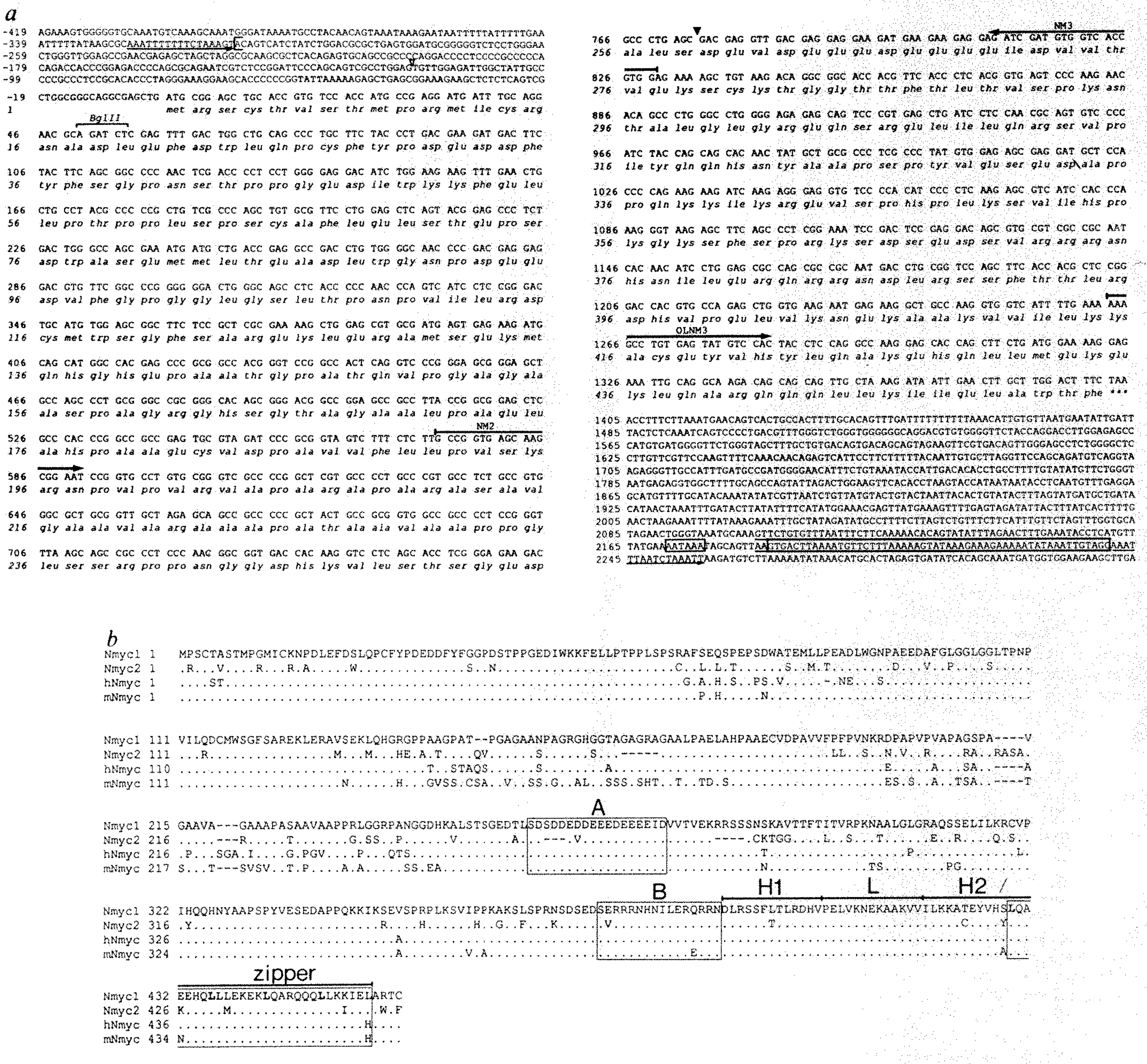

FIG. 2 Nucleotide sequence of N-*myc2* and N-Myc protein sequence comparison. *a*, The putative coding region of N-*myc2* has been translated into amino acids beneath the corresponding nucleotides; numbering is from the first ATG codon. The region showing homology with N-*myc1* exonic sequences is bracketed. The analogous locations of the first and second N-*myc1* introns are indicated by an open and a black arrowhead, respectively. Flanking direct repeats are underlined with an arrow. The polyadenylation signal and the downstream adenosine-rich sequence stemming from a poly(A)$^+$ tail are boxed. Sequences overlined with arrows show oligonucleotides used as primers in reverse transcriptase-PCR experiments. NM2 and NM3 were used for the identification of the 2.3-kb N-*myc2* RNA produced in most tumours from an unrearranged allele. The sequencing strategy was as reported in Fig. 1. *b*, The predicted amino-acid sequences of the proteins encoded by the woodchuck N-*myc1*, N-*myc2*, human N-*myc*[8] (hNmyc) and murine N-*myc*[9] (mNmyc) genes are shown aligned for maximum homology. Amino acids are represented with the single-letter code only when different from the residue found at the homologous position in N-Myc1 and otherwise by a dot when identical, or a dash when deleted. The acid and the basic motifs are boxed (A and B, respectively), the helix-loop-helix structure overlined (H1-L-H2-zipper). The 'leucine zipper' is also boxed with the critical leucines shown in bold character. The N-Myc2 acid motif, shortened compared with the other N-Myc proteins, is quite similar to that of c-Myc. The predicted N-Myc1 polypeptide shows 90%, 86% and 80% homology with human N-Myc, murine N-myc and N-myc2 proteins, respectively.

activated in liver tumours, either by downstream insertion of WHV or in the absence of genetic rearrangements. This cDNA gene stands presently as one of the few cases in which processed copies of intron-containing genes have evolved into functional genes[26,27]. The intronless rat s-*myc* gene, which has lost the transforming capacity of the parental *myc* gene and acquired a suppressive effect on tumorigenicity, is a seemingly related example[28]. In this context, it will be interesting to determine whether N-*myc2* is expressed in the developing embryo or in adult woodchuck tissues, and whether it has acquired some specific function in normal cells. In addition, together with the activation of c-*myc* previously described[1], we report insertional

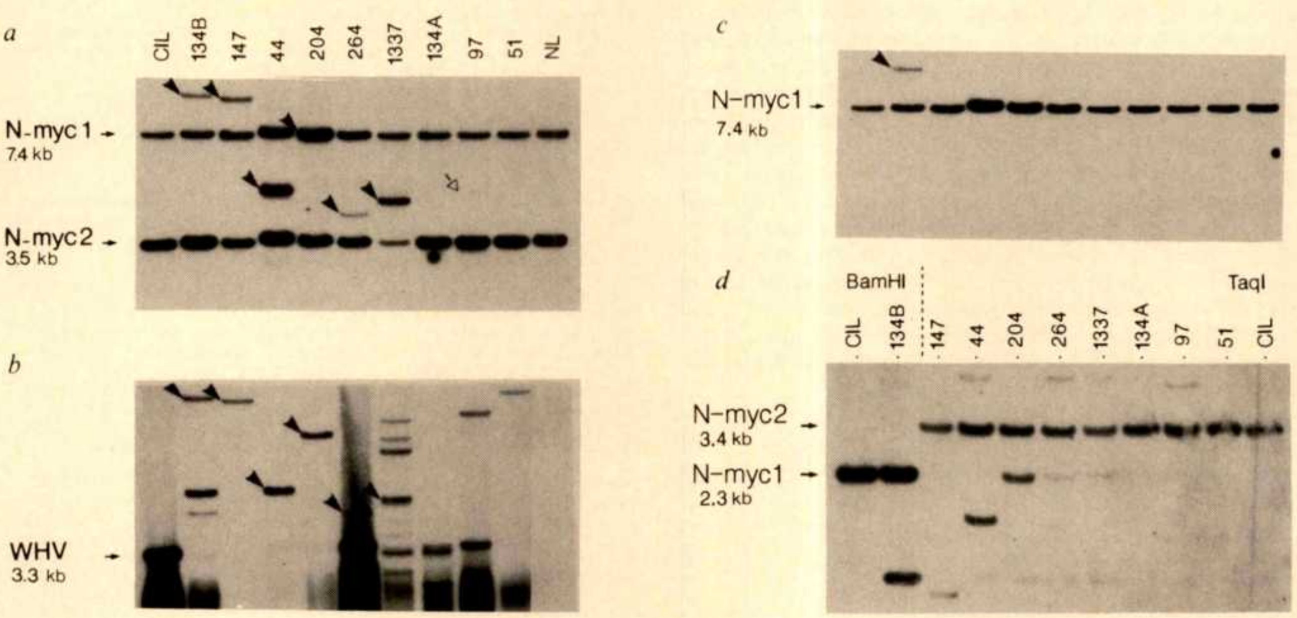

FIG. 3 Integration of WHV DNA into N-*myc* genes in woodchuck HCC. *a*, *b* and *c*, Southern blot analysis of *Hin*dIII-digested DNA from nine woodchuck HCCs (named above each lane), a WHV-infected liver bearing no HCC (CIL) and a normal liver (NL), hybridized sequentially with the N-*myc* probe M13.13 (*a*), the viral subgenomic fragment WHV En (*b*) and the N-*myc1*-specific probe SpIntI (*c*). Solid arrowheads indicate tumour-specific DNA bands detected by the N-*myc* and WHV probes. Open arrow marks the W97 HCC-specific N-*myc2* band that failed to hybridize with the viral probe. Viral replicative forms are seen in (*b*) as smears beneath the indicated position of the double-stranded WHV genome. Fragments of higher relative molecular mass represent viral sequences integrated into the cellular genome. 134A and B were two different tumour nodules on the same woodchuck liver. *d*, Southern blot showing DNA rearrangements in the third exon of either N-*myc1* (in W134B HCC) or N-*myc2* (in six other tumours). N-*myc2* bands that are fainter than the N-*myc1* bands reflect incomplete annealing of the N-*myc1* TqEx3C probe to N-*myc2* exon 3 sequences at high stringency.

METHODS. Total DNA was prepared[1] from frozen liver and tumour samples, digested with different restriction enzymes, separated by size (20 μg DNA per lane), blotted in alkali to Hybond N$^+$ membranes (Amersham) and hybridized with randomly labelled probes as described[38]. WHV En is a 612-bp *Apa*I–*Nco*I fragment (position 888–1,500). After hybridization with TqEx3C, washings were performed at higher stringency (0.08 SSC buffer, 0.1% SDS, 65 °C) to minimize annealing of the probe to *myc* sequences different from N-*myc*. The filters were exposed at −80 °C overnight.

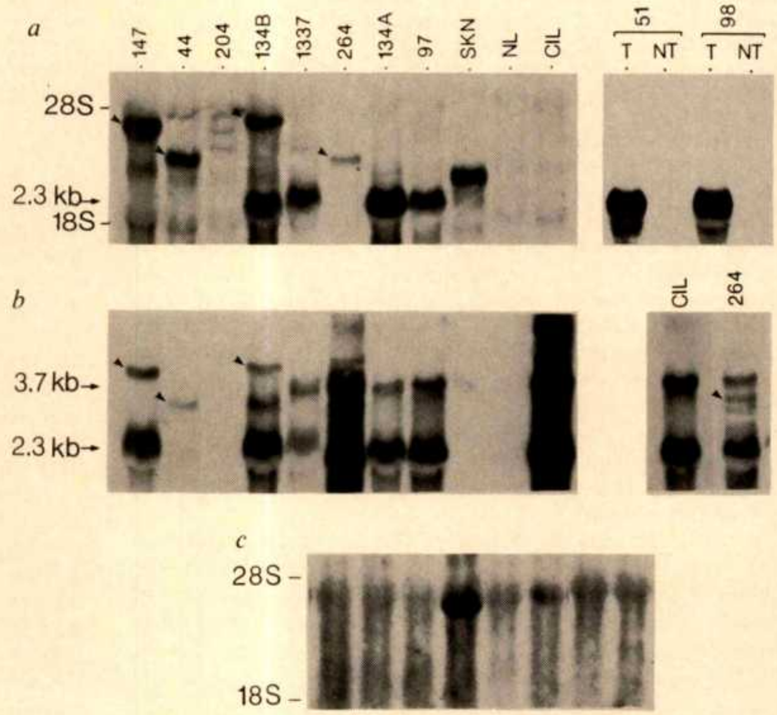

FIG. 4 Altered N-*myc* expression in woodchuck HCC. *a*, Northern blot of liver and tumour RNA, prepared from the biopsy samples also used for DNA analysis (Fig. 3), from the tumorous (T) and non-tumorous (NT) parts of two livers (W51 and W98) showing no rearrangements of N-*myc* genes (W98 HCC DNA not shown) and from the human neuroblastoma cell line SKN-B2. The M13.13 probe detects N-*myc1* and N-*myc2* transcripts. *b*, Same blot probed with WHV En. The sizes of the normal WHV transcripts are indicated. Shorter exposures are shown for W264 HCC and WHV-infected liver RNAs in the right lanes. *c*, Northern blot hybridization with the N-*myc1*-specific probe SpInt1. RNA samples were as in the first eight lanes of *a* and *b*. Only the 4.5-kb RNA in W134B HCC is detected. Positions of 28S and 18S ribosomal RNAs indicated.

METHODS. Total RNA was prepared and analysed as previously described[1]; 40 μg RNA was loaded per lane, except for SKN-B2 RNA (10 μg). The filters were exposed at −80 °C for 3 days (N-*myc* hybridization) or for 6 h (WHV hybridization).

mutagenesis of *myc* genes by WHV DNA in about one third of the hepatocellular carcinoma examined (10% c-*myc*; 20% N-*myc*). These results clearly establish that woodchuck hepatitis virus, like nonacute retroviruses, contributes to neoplastic transformation through insertional activation of cellular proto-oncogenes. WHV is closely related to the human hepatitis B virus (HBV) and serves as a model for liver disease and for hepatocarcinogenesis in humans. Woodchuck hepatomas, however, differ notably from a majority of human hepatomas in the earlier onset of tumours in the carrier state and in the absence of associated cirrhosis, suggesting that HBV might act more indirectly. Transactivation of cellular genes by the truncated products of integrated HBV preS2/S genes[29] and by the X protein[30], and long-term storage of the viral glycoprotein encoded by the pre-S1/pre-S2/S region[31] have been proposed as causative factors in HBV-associated oncogenesis. HBV integration near *myc* genes or other currently known proto-oncogenes has not been observed thus far; however, the finding of HBV insertion sites within retinoic acid receptor β and cyclin A, two genes essential for cell growth and differentiation, in two independent tumours[32,33], supports the idea that HBV may act, at least occasionally, as an insertional mutagen. ☐

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Relationship between the product of the *Drosophila ultraspiracle* locus and the vertebrate retinoid X receptor

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THE vitamin A derivative, retinoic acid, can regulate morphogenesis and differentiation in vertebrates[1,2]. Two different subfamilies of the steroid receptor superfamily of transcription factors, the retinoic acid receptors and the retinoid X receptor, mediate the effects of retinoic acid[3–6]. As part of an analysis of the hormonal control of development, we have examined the *Drosophila* genome for retinoic acid receptor homologues[7]. Here we describe one such gene, *XR2C*, which encodes a product with structural similarity to the human retinoic acid-responsive transcription factor, retinoid X receptor. This receptor-like protein is encoded by *ultraspiracle* (*usp*), a locus required both maternally and zygotically for pattern formation. The discovery that the *usp* product is a retinoid X receptor homologue suggests that similar chemical cues underlie morphogenic signalling in vertebrate and invertebrate systems.

Potential homologues of vertebrate steroid hormone receptor-like genes were identified by probing a blot of wild-type *Drosophila* genomic DNA with DNA encoding the human retinoic acid receptor (hRARα) DNA-binding domain. The genes corresponding to the four darkest bands were cloned, sequenced and cytologically mapped[8], but none of the isolates showed greater than 60% sequence identity to the *RARα* in the DNA-binding domain. One class of isolates maps to cytologic position 2C on the X chromosome (not shown) and was called *XR2C*. A 300-base pair (bp) *Pst*I-*Bam*HI fragment (PB), encoding the *XR2C* putative DNA-binding domain (Fig. 1*a*), hybridizes to a single fragment in both *Eco*RI- and *Xho*I-digested genomic DNA (Fig. 1*b*), demonstrating that *XR2C* is a single-copy gene. Further, the PB probe recognizes a few cross-hybridizing bands on Southern blots of vertebrate genomic DNA (Fig. 1*c*), indicating that *XR2C*-related sequences are conserved throughout the vertebrate lineage. Although *XR2C* was isolated with h*RARα* probe, the bands that cross-hybridize with *RARα* gave a signal below detection in the human lane. Instead, the pattern of hybridization is the same as that seen for the human retinoic acid-responsive transcription factor, retinoid X receptor (RXRα) (see below)[6].

The structure of the *XR2C* transcripts was determined from the sequence of complementary DNA clones isolated by screening larval and pupal cDNA libraries with the PB probe. The predicted protein product reveals a marked similarity with the hRXR. The nucleotide sequence of the longest insert is shown in Fig. 2*a* and a comparison of the predicted amino-acid sequence of XR2C with that of other nuclear receptors in both vertebrates and *Drosophila*, is shown in Fig. 2*b*, *c*. XR2C is 86% identical to RXRα in the DNA-binding domain but only 59% in the same region of the hRARα. XR2C shares 49% identity in the hormone-binding domain with RXR and only 24% with the RAR. These results confirm the evolutionary conservation of the RXR subfamily.

Comparison of the XR2C restriction map to that of a previously published genomic walk of the 2C–2E region, oriented the *XR2C* transcript to the chromosome and to several key deletion breakpoints[9]. Genetic characterization of lethal mutations relative to these breakpoints identified three possible candidate genes for *XR2C*: *l(1)2Cc*, *l(1)2Ce* and *ultraspiracle* (*usp*)[10,11]. To assign a locus to the *XR2C* gene, blots of genomic DNA from alleles of these genes were examined for associated rearrangements in the *XR2C* transcription unit.

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Only one mutant, usp^{KA21}, was associated with a rearrangement in *XR2C* (not shown). To localize the precise breakpoint relative to the *XR2C* coding region, a genomic library from heterozygous usp^{KA21} females was constructed and screened with the 8-kilobase (kb) *Eco*RI fragment that contains *XR2C* and spans the breakpoint. Analysis of the structure of clones containing the breakpoint reveals the *XR2C* coding region has been disrupted within the putative DNA-binding domain (Figs 1*a* and 2*a*). The region flanking the *XR2C* transcription unit seems to be unaltered. These data show that *XR2C* is mutated on the usp^{KA21} chromosome and suggest that *XR2C* corresponds to the *usp* locus.

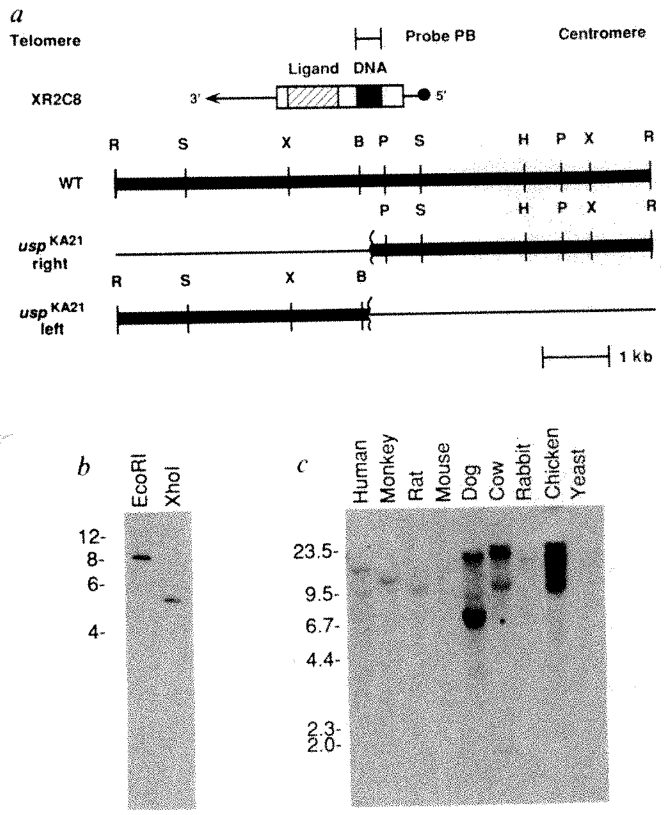

FIG. 1 Structure, copy number and evolutionary conservation of *XR2C*. *a*, Schematic diagram of the structure of the *XR2C* locus. A restriction map of the wild-type (WT) genomic region is shown as a thick line. The structure and extent of the transcription unit is indicated above the genomic map, as is the position of the *Bam*HI–*Pst*I fragment used as hybridization probe (probe PB). All of the cDNAs isolated were colinear with the genomic sequence, as determined by DNA sequencing and restriction mapping, indicating that there are no intervening sequences. The lengths of the cDNAs are consistent with the estimated RNA size of 2.4 kb (see Fig. 3). The structure of the cloned breakpoints of the usp^{KA21} genomic DNA is shown beneath the wild-type restriction map. Thick lines, *XR2C* DNA; thin lines, non-*XR2C* DNA. Sequence analysis indicates that the usp^{KA21} breakpoint occurs in the region coding for the second finger of the putative DNA-binding domain of *XR2C* at nucleotide positions 661–663 within codon Cys 167 (see Fig. 2*a*). Restriction enzymes: R, *Eco*RI; S, *Sal*I; X, *Xho*I; B, *Bam*HI; P, *Pst*I; H, *Hin*dIII. *b*, Southern blot of Canton S wild-type DNA digested with either *Eco*RI or *Xho*I was probed at high stringency with PB and reveals that *XR2C* is a single copy gene that lies on an 8.0-kb *Eco*RI and 4.5-kb *Xho*I fragment. *c*, Southern blot of genomic DNAs from various species was probed at low stringency with PB and reveals the existence of a few cross-hybridizing species in all organisms tested except for yeast. In the human lane, two main bands appear, of about 15 and 9 kb. These bands correspond to the bands seen when the DNA-binding domain of hRXRα is used as a probe[6] DNA size markers (kb) to the left.

METHODS. Standard methodology was used for isolation of genomic DNA and Southern blotting[18] and conditions for high and low stringency hybridization were as described elsewhere[19]. The Southern blot of genomic DNA from various species was purchased from Clontech (Palo Alto) and probed under low stringency conditions[19]. The *usp* library was made by digesting 10 μg genomic DNA from $usp^{KA21}/FM7$ females, size-fractionating the resultant fragments on an agarose gel, and isolating fragments of sizes 2–7 kb by electroelution. The DNA was packaged using LambdaZap II, *Eco*RI-digested phage arms(Stratagene). Phage were screened with a labelled 8-*kb* *Eco*RI fragment and plaque-purified candidate phage were converted to plasmids by the addition of helper phage R408 (Stratagene).

TABLE 1 Number of flies

Allele	$\frac{usp}{y}$; $\frac{p[XR2C^+]}{+}$	$\frac{usp}{y}$; $\frac{TM3}{+}$	$\frac{usp}{+}$; $\frac{p[XR2C^+]}{+}$	$\frac{usp}{+}$; $\frac{TM3}{+}$	Relative viability
usp^{VE653}	154	0	128	116	1.2
usp^{VE849}	126	0	124	90	1.0
usp^{KA21}	100	0	140	87	0.7

XR2C genomic region specifically complements *usp* mutants. Complementation tests were performed by crossing XX *usp/FM7* (ref. 14) females to +/Y; p[XR2C$^+$]/*TM3* males. Surviving *usp*/Y flies were scored with regard to their third chromosome genotype. Numbers indicate number of mutant flies that survived to adults. Columns are as marked. Rows indicate the particular *usp* allele tested. The alleles of other loci tested[11] were *l(1)2Cc*^{2}, *l(1)2Ce*^{1}, *l(1)2Ce*^{2}, and *l(1)2Ce*^{3}. Alleles from the *l(1)2Cb* complementation group had previously been shown to be outside the genomic walk and were not tested (E. Fyrberg, personal communication). Relative viability was measured by comparing the number of rescued mutant males (*usp*/Y;p[XR2C$^+$]/+) with the number of transposon-containing *usp*$^+$ siblings (*usp*/+, p[XR2C$^+$/+]). The relative viability of the mutant males with the transposon was near unity for each allele. The P-element germ-line transformation vector is pUChsneo (ref. 15) and contains the 8-kb *Eco*RI fragment inserted into the polylinker. G1 progeny of surviving, injected embryos were slected on *Drosophila* instant food containing 250 μg ml^{-1} G418 as described previously[16]. One line shown to contain an insert by Southern analysis and resistance to G418 had an insert that was mapped by segregation to the third chromosome and balanced over *TM3*. Mutations in the 2C region had been generated previously[17]. Complementation was performed by crossing XX female flies of genotype *usp/FM7*; +/+ with males of genotype +/Y;p[XR2C$^+$]/*TM3* in vials on standard corn meal, molasses, agar media[18]. Parental flies were placed on new food every 3 days.

To test this hypothesis, the 8-kb *Eco*RI genomic fragment (see Fig. 1*a*) containing the *XR2C* gene was placed, by germ-line transformation, onto a wild-type third chromosome. Complementation tests were performed by crossing the wild-type copy of *XR2C* into mutant backgrounds and scoring for survival of the otherwise lethal class of flies. The 8-kb *Eco*RI fragment of *XR2C* specifically complements the three *usp* alleles (Table 1), but not four alleles tested from other complementation groups at position 2C (data not shown). The relative viability of the rescued flies is close to unity for all three alleles, indicating that the 8-kb genomic fragment containing the *XR2C* transcription unit is sufficient for *usp* function.

The *usp* locus has been previously characterized for its zygotic and maternal germ-line functions[10]. Removal of both maternal germ-line and zygotic *usp* expression results in an embryonic segmental abnormality involving a cuticular defect posterior to the eighth abdominal denticle belt. A zygotic wild-type copy of *usp* rescues the phenotypic consequences of a lack of maternal *usp* product and results in apparently normal development. Removal of zygotic expression from the progeny of *usp*/+ mothers results in a milder defect. These *usp*/Y embryos die as early second instar larvae with an additional set of posterior spiracles and filzkorper, thought to be due to the persistence of cuticular structures from earlier molts. Because of the epistasis of early death over other phenotypes, it has not been determined if this gene is needed for later functions. As a first step towards determining whether *usp* might be required at later stages, we examined the developmental profile of *usp* RNA expression (Fig. 3). Northern analysis reveals a single 2.4-kb transcript throughout the life cycle, with highest levels in adults and

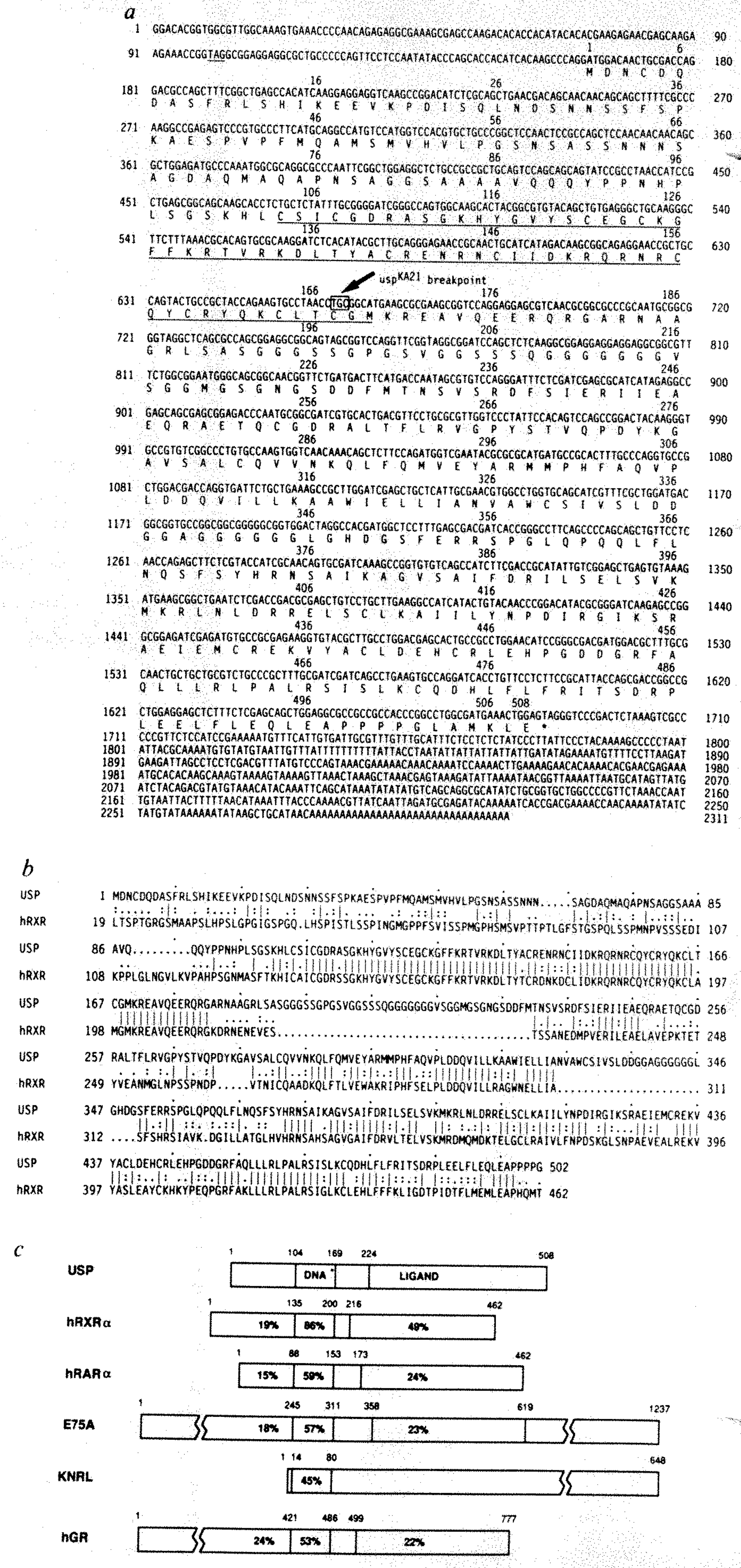

FIG. 2 The nucleotide and predicted amino-acid (single-letter code) sequences of XR2C compared with the predicted amino-acid sequence of other members of the nuclear receptor superfamily. *a*, The complete nucleotide sequence of pXR2C8, the longest of five independent cDNA clones isolated by screening third instar larval imaginal disc and pupal cDNA libraries with the PB probe at high stringency. The predicted amino-acid sequence starts with a methionine at nucleotide 163 and ends with a stop codon at nucleotide 1,689. The initiator methionine conforms to the *Drosophila* consensus sequence for translation initiation[20]. An upstream, in-frame stop codon is underlined, implicating the first AUG codon as the start of initiation. Also underlined is the putative DNA-binding domain. The breakpoint of *usp*^{KA21} occurs within the putative DNA-binding domain at the codon for Cys 167 (arrow) and results in a deletion of nucleotide positions 661–663 (boxed nucleotides). Further, the chromosomal break juxtaposes a stop codon immediately after the codon for Thr 166. *b*, Alignment of the predicted amino-acid sequences of the *usp* and h*RXRα* protein products. Maximal alignment was determined using the local homology algorithm of Smith and Waterman on the BESTFIT program[21]. Symbols used for amino-acid comparisons are: (—), amino acid identity; (:), close evolutionary relationship; (.), distant relationship; no symbol, very distant relationship as defined by Dayhoff and normalized by Gribskov[22]. Note the high identity in the DNA-binding domain and in clusters of the putative ligand-binding domain. The *usp* amino-acid sequence contains two insertions of glycine-rich sequences before and in the middle of the putative ligand-binding domain (amino-acid positions 192–232 and 325–350). *c*, Comparisons of the predicted amino-acid sequence of the *usp* product with the predicted sequences of other nuclear receptors from both vertebrates and invertebrates. The region marked 'DNA' is compared with the 66–68-amino-acid DNA-binding domain and the region marked 'Ligand' is compared with the region following the DNA-binding domain which shares the greatest alignment with other nuclear receptors. The carboxy-terminal sequence of KNRL shares no significant sequence similarity with other receptors and, consequently, no alignment was attempted. Note the striking similarity in the DNA-binding and putative ligand-binding domains between the *usp* product and hRXR, much greater than that between other identified receptors.

METHODS. Screening, isolation and sequencing of candidate cDNAs were performed as described elsewhere[23]. The sequence data were assembled and comparisons were performed using programs of Devereux *et al.*[21]. Numbers indicating amino acids are as detailed from USP (this report), hRXRα (ref. 6), hRARα (ref. 4), E75A (ref. 24), KNRL (ref. 8), and hGR (ref. 25).

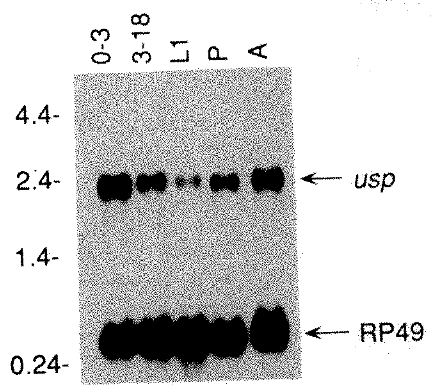

FIG. 3 Developmental expression of *usp*. A northern blot of poly(A)$^+$ mRNA isolated from different stages of the *Drosophila* life cycle was probed with PB and revealed that *usp* gives rise to a single class of RNA of 2.4 kb throughout the *Drosophila* life cycle. The levels of the 2.4-kb RNA are roughly equivalent at all stages examined. Lanes 0-3 and 3-18 contain RNA extracted from embryos collected 0-3 and 3-18 h after egg laying, respectively. L1, RNA from first instar larvae; P, RNA from pupae; and A, RNA from adult males and females. As a quantitative control, the blot was stripped and reprobed with labelled DNA coding for the ribosomal protein RP49 which is expressed at roughly constant levels throughout development[26]. RNA size markers (kb) to the left.
METHODS. The staged organisms were collected and *Drosophila* mRNA was isolated and poly(A)-selected by standard methods[18]. Poly(A)$^+$RNA 10 μg were loaded per lane.

embryos and lower levels in larvae. Expression of *usp* after the lethal phase of *usp* mutants suggests a continuing role for *usp* through many developmental stages.

We demonstrate that an RXR-like molecule plays an important part in embryonic and post-embryonic development. The marked similarity between the products of the *usp* and h*RXR* genes indicates that the RXR subfamily is of ancient origin, existing before the divergence of insects and vertebrates. The inability to isolate a closely related RAR gene may reflect fortuitous restriction enzyme sites in the gene sequence or, more likely, an earlier origin of the RXR subfamily or greater subsequent rate of divergence of the RAR genes. We have demonstrated here that at least one member of the RXR subfamily is necessary for proper development of a metazoan organism. The homology between the DNA-binding domains of the *RXR* and *usp* gene products suggests that these proteins recognize similar DNA sequences. The similarity in the putative ligand-binding domain is roughly the same as that between the human glucocorticoid and mineralocorticoid receptors, which respond to an overlapping set of ligands[12]. Metabolic studies indicate that *Drosophila* do not require vitamin A or its precursor β-carotene except for visual function and that they are incapable of synthesizing them *de novo*[13]. Moreover, preliminary studies using a cotransfection assay in Schneider cells, developed to characterize the vertebrate homologue RXRα[6], indicate that the product of the *usp* gene does not activate transcription in response to retinoic acid (data not shown). Together these data suggest that the critical endogenous ligand to which the *usp* gene product responds is not retinoic acid but may be a structurally similar molecule synthesized by an alternative metabolic pathway. ☐

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Studying single living cells and chromosomes by confocal Raman microspectroscopy

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MANY indirect methods have been developed to study the constitution and conformation of macromolecules inside the living cell. Direct analysis by Raman spectroscopy is an ideal complement to techniques using directly labelled fluorescent probes or of indirect labelling with mono- and polyclonal antibodies. The high information content of Raman spectra can characterize biological macromolecules both in solution and in crystals[1,2]. The positions, intensities and linewidths of the Raman lines (corresponding to vibrational energy levels) in spectra of DNA–protein complexes yield information about the composition, secondary structure and interactions of these molecules, including the chemical microenvironment of molecular subgroups. The main drawback of the method is the low Raman scattering cross-section of biological macromolecules, which until now has prohibited studies at the level of the single cell with the exception of (salmon) sperm heads, in which the DNA is condensed to an exceptionally high degree[3]. Ultraviolet-resonance Raman spectroscopy has been used to obtain single cell spectra (ref. 4; and F. Sureau and P. Y. Turpin, personal communication), but in this method absorption of laser light may impair the integrity of the sample. We have avoided this problem in developing a novel, highly sensitive confocal Raman microspectrometer for nonresonant Raman spectroscopy. Our instrument makes it possible to study single cells and chromosomes with a high spatial resolution ($\lesssim 1\ \mu m^3$).

The configuration of our instrument (fig. 1) was carefully optimized to obtain a maximal signal throughput (~15% of the scattered light collected by the microscope objective is actually detected). Light detection by the liquid-nitrogen-cooled charge-coupled-device camera is virtually photon-noise limited. Incident light of 660 nm is used to prevent degradation of the sample in the focused beam, a phenomenon observed with chromosomes and cells using the 514.5-nm line of an Argon-ion

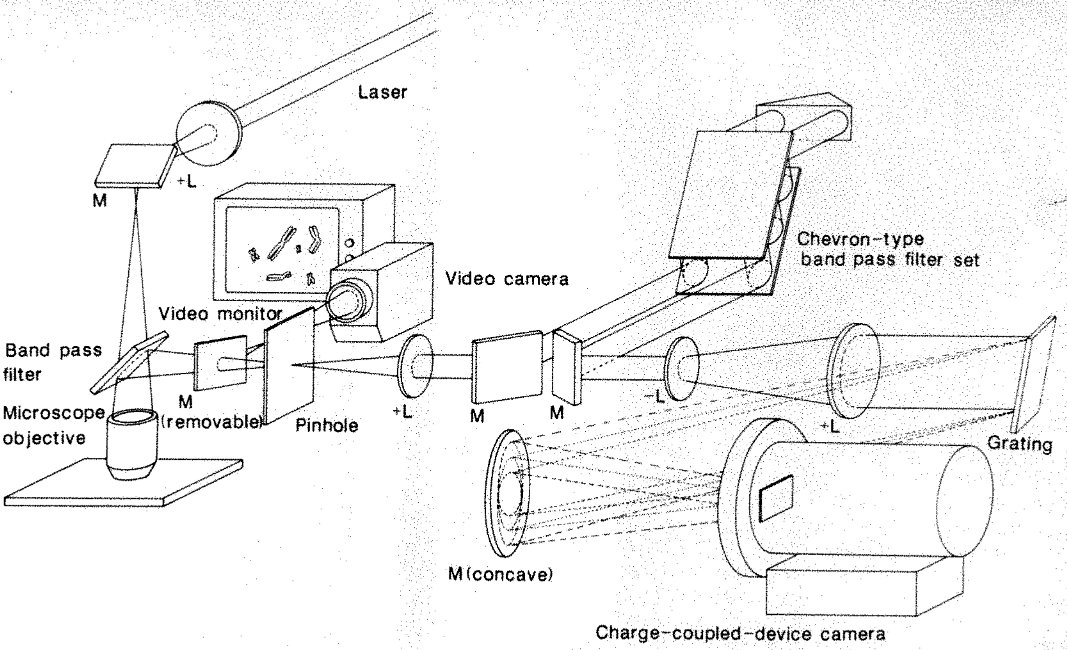

FIG. 1 Confocal Raman microspectrometer. Laser light of 660 nm from a DCM operated model 375 B Spectra Physics dye laser is focused on the object under investigation by a high numerical aperture microscope objective. Light scattered by the object is collected by the same objective, and coupled into the spectrometer through a pinhole, which enables confocal detection[10]. Lateral spatial resolution in the CRM is determined by the laser focus dimensions and is ≤0.5 μm. A pinhole of 100 μm diameter leads to a depth resolution of 1.3 μm. The spectrometer consists of a chevron-type dielectric band-pass filter set, which combines efficient stray light suppression (by a factor 10^9) with a high transmission (80–90% in the spectral interval 600–1,800 cm^{-1}) for Raman light (G. J. Puppels, A. Huizinga, H. W. Krabbe, H. A. de Boer, G. Gijsbers and F. F. M. de Mul, manuscript in preparation), and a wavelength dispersion stage. A liquid-nitrogen-cooled charge-coupled device camera (equipped with an English Electric Valve Co. P8603 B charge-coupled device chip, Wright Instruments Ltd) is used for signal detection. It combines a high quantum efficiency (~40% at 700 nm) with almost noise-free operation (negligible dark-current and a read-out noise of r.m.s. 10 electrons, equalling 10 detected photons). The spectral resolution of the microspectrometer is ~6–7 cm^{-1}. Abbreviations: M, mirror; L, lens.

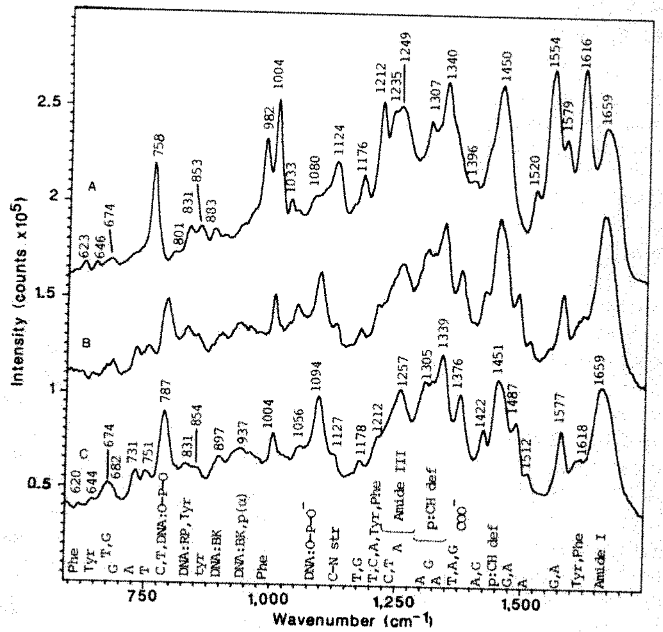

FIG. 2 Raman spectra of a single intact cell and a single metaphase chromosome. Spectrum A obtained from a cytoplasmic region of a human eosinophilic granulocyte. Spectrum B from the nucleus of the same cell, C obtained from a chromatid of a metacentric Chinese hamster lung cell (V79) metaphase chromosome. Intensity scale is for A (for B it is multiplied by 5, for C by 1.25). Spectra shifted along the ordinate for clarity. Raman line assignments are for nucleus and chromosome spectrum (B, C) and based on assignments in[5,6,11–13]. Water (A, B, C) and substrate (C) signal subtracted. Abbreviations: T, thymine; C, cytosine; A, adenine; G, guanine; RP, ribophosphate; BK, backbone; p, protein; α, α helix; Tyr, tyrosine; Phe, phenylalanine; def, deformation; str, stretching. Experimental conditions: ×63 Zeiss Plan Neofluar water-immersion objective (NA 1.2); laser power 5 mW (A,B), 10 mW (C); measuring times 150 s (A, B) or 900 s (*c*); granulocyte on poly-L-lysine-coated fused silica (fs) substrate in standard PBS isolation method of ref 14; chromosome on fs substrate in hypotonic buffer (4 mM $MgCl_2$, 10 mM Tris and 10 mM NaCl), isolation method of ref. 15.

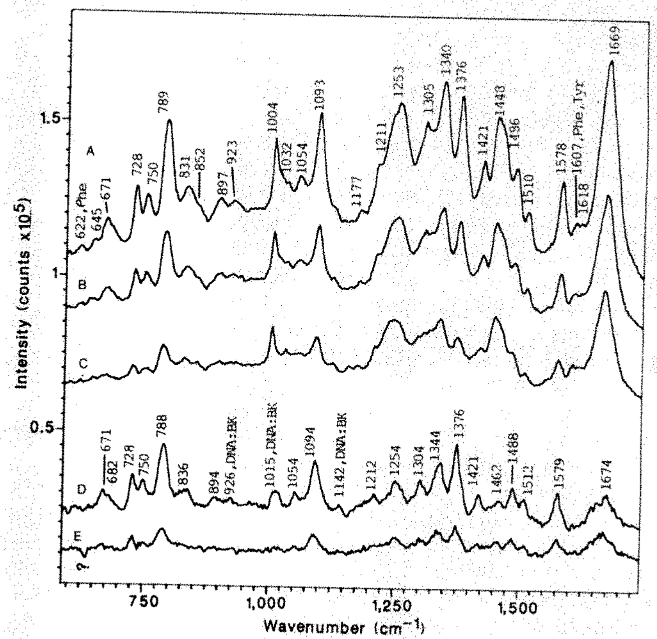

FIG. 3 Raman spectra obtained from polytene chromosome II of *Chironomus thummi thummi* (salivary gland), shown in Fig. 4. Spectrum A, band (arrow 1); B, interband (arrow 2); C, telomere (arrow 3); D, difference spectrum A – C scaled on 1450 cm^{-1} protein line; E, difference spectrum A – B (as in D, multiplied by 2). Spectra shifted along ordinate for clarity. Raman line assignments as in Fig. 2 spectra B and C. The line marked '?' is most probably artefactual and caused by detection of a cosmic ray event in the recording of spectrum B. Buffer signal subtracted (A, B, C). Experimental conditions: ×63 Zeiss Plan Neofluar water-immersion objective (NA 1.2/0.12–0.22); laser power, 15 mW; measuring time, 600 s; chromosome on fs substrate in PBS with added divalent cations (1 mg ml^{-1} $MgCl_2$ and 1 mg ml^{-1} $CaCl_2$) covered by fs coverslip. Fixation procedure according to ref. 8.

laser (G. J. Puppels, J. H. F. Olminkhof, G. M. J. Segers-Nolten, C. Otto, F. F. M. de Mul and J. Greve, manuscript in preparation).

The data reported here exemplify the spatial resolution and sensitivity of the microspectrometer. The spectra shown are not smoothed and were recorded with the samples at room temperature. Figure 2 shows spectra obtained from the cytoplasm (*a*) and the nucleus (*b*) of an intact human eosinophilic granulocyte. The nucleus spectrum consists of lines that can be assigned to DNA and protein vibrations and strongly resembles reported spectra of isolated chromatin[5,6] and also spectrum 2C, which was obtained from a single metaphase chromosome. The cytoplasm spectrum differs dramatically from that of the nucleus. This is most probably due to signal contributions from the many cytoplasmic granules, the Raman lines of which have so far to be assigned. The amount of DNA present in the measuring volume during the recording of spectrum 2C was estimated to be ~50 fg, corresponding to 50×10^6 base pairs. The high quality of the spectrum indicates that even much smaller amounts of material would suffice for recording good spectra.

The experiments with metaphase chromosomes are aimed at obtaining a better understanding of the origins of the banding patterns that appear after physico-chemical treatment and staining as first described by Caspersson *et al.*[7]. Figure 3 displays spectra obtained from a band (*a*), an interband (*b*) and a telomere (*c*) of a fixed polytene chromosome (figure 4). An interesting feature is the difference in the DNA/protein ratio between bands, interbands and telomeres. Figure 3*d*, *e* shows difference spectra scaled on the 1,450 cm^{-1} protein line. From the fact that only DNA lines remain, it is clear that this ratio is lowest in the telomere and highest in the bands. Whereas spectra obtained from different chromosome bands are very similar both in absolute signal intensity and relative intensity of the Raman lines, spectra from different interbands show marked variations, indicative of different DNA-protein ratios. In all cases this ratio is lower in interbands than in bands. We are presently investigating the possibility that these differences are related to local variations in transcriptional activity, by studying unfixed chromosomes as well. The microspectrometer is also being employed to study the presence and induction of left-handed Z-DNA in such chromosomes[8,9].

The use of 660-nm laser light enables Raman measurements on cells and chromosomes which have been labelled with fluorescein-conjugated antibodies, thus facilitating the prior localization of specific structures such as sites of transcription or replication by conventional fluorescence microscopy. The combination with (immuno)fluorescence techniques thus further increases the potentials of the CRM. Moreover the Raman spectra could yield valuable information about the way samples (and experimental results) may be affected by the application of fluorescence techniques.

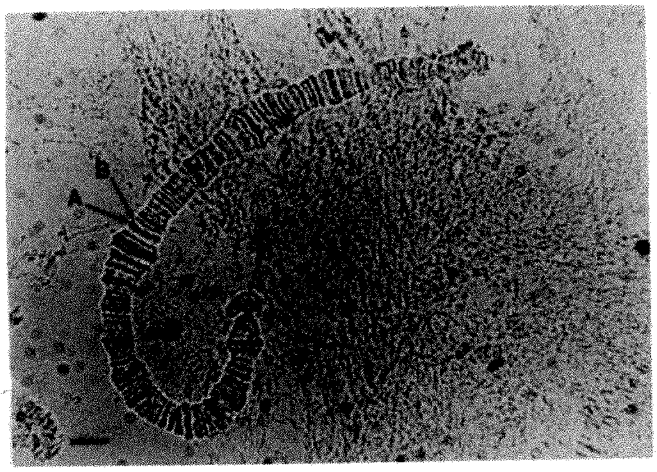

FIG. 4 Polytene chromosome II of *Chironomus thummi thummi* (salivary gland). Indicated are the positions where the spectra of Fig. 3 were obtained. Bar, 10 μm.

Finally, we expect a vast range of applications of this instrument in the analysis of small biological structures and possibly for monitoring biological events, such as the cytotoxic process mediated by natural killer cells, on a single cell level. ☐

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Histidine oxidation in the oxygen-evolving photosystem-II enzyme

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THE evolution of oxygen as a result of light-driven water oxidation occurs in plants and is catalysed by photosystem-II (PS-II). A manganese-cluster probably acts both as the active site and as a charge-accumulating device (for a review, see ref. 1). The enzyme cycle involves five redox states which are denoted as S_0–S_4, depending on the number of positive equivalents stored[2]. Oxygen is released after formation of the transient S_4 state. Ca^{2+} is an obligatory cofactor in this process and its depletion inhibits the enzyme cycle at the step before water oxidation, that is, after formation of the S_3 state[3]. In chelator-treated, Ca^{2+}-depleted PS-II, a new electron paramagnetic resonance (EPR) signal arising from a formal S_3 state has been reported[4]. It was suggested that the S_3 EPR signal could originate from the oxidation of an amino acid, interacting magnetically with the manganese cluster[4]. There are only a few examples of amino-acid oxidation in enzyme chemistry and these are limited to tyrosine[5] and tryptophan[6]. Here we report evidence that the S_2 to S_3 transition occurring in Ca^{2+}-depleted PS-II corresponds to the oxidation of histidine.

As reported previously[4], we observed two new EPR signals in Ca^{2+}-depleted PS-II. First, a signal at $g=1.98$ consisting of many lines (>26) spread over 1,700 gauss which is stable in the dark at 0 °C and is attributed to a modified S_2 state. This multiline signal is a modified form of that arising from the S_2 state in the active enzyme[7] and it is likewise ascribed to a mixed-valence manganese cluster having a spin-$\frac{1}{2}$ ground state[4]. The second new EPR signal, which is attributed to a formal S_3 state, is centred at $g=2.004$, with a linewidth of 164 G. Previously we

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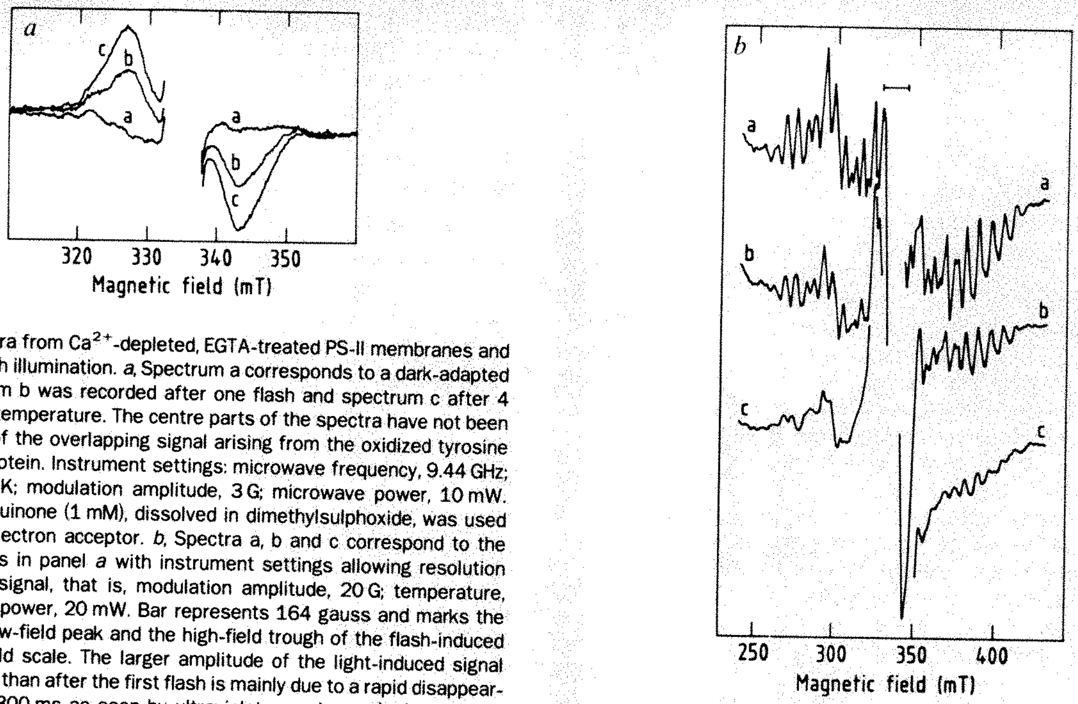

FIG. 1 EPR spectra from Ca^{2+}-depleted, EGTA-treated PS-II membranes and the effect of flash illumination. *a*, Spectrum a corresponds to a dark-adapted sample. Spectrum b was recorded after one flash and spectrum c after 4 flashes at room temperature. The centre parts of the spectra have not been drawn because of the overlapping signal arising from the oxidized tyrosine 161 of the D_2 protein. Instrument settings: microwave frequency, 9.44 GHz; temperature, 15 K; modulation amplitude, 3 G; microwave power, 10 mW. Phenyl-*p*-benzoquinone (1 mM), dissolved in dimethylsulphoxide, was used as an artificial electron acceptor. *b*, Spectra a, b and c correspond to the same samples as in panel *a* with instrument settings allowing resolution of the multiline signal, that is, modulation amplitude, 20 G; temperature, 10 K; microwave power, 20 mW. Bar represents 164 gauss and marks the position of the low-field peak and the high-field trough of the flash-induced signal on this field scale. The larger amplitude of the light-induced signal after four flashes than after the first flash is mainly due to a rapid disappearance (half life ~300 ms as seen by ultraviolet experiments) of the signal in ~40% of PS-II centres during the time between flash and freezing of the sample. This disappearance is due in part to charge recombination with the electron stored on the acceptor side. Therefore four flashes are required to trap almost 100% of the light-induced state.

METHODS. PS-II membranes were depleted of Ca^{2+} by a treatment with NaCl and EGTA (ref. 4). The procedure used maintains the polypeptide constitution of PS-II. EPR spectra were recorded with a Bruker ESP 300 X-band spectrometer equipped with an Oxford Instrument cryostat. Flash illumination was as in ref. 3. The time required to freeze the EPR sample after flash excitation was about 2 s. Concentration of the sample was ~2.5 mg chlorophyll per ml in 0.3 M sucrose, 10 mM NaCl, 25 mM MES pH 6.5.

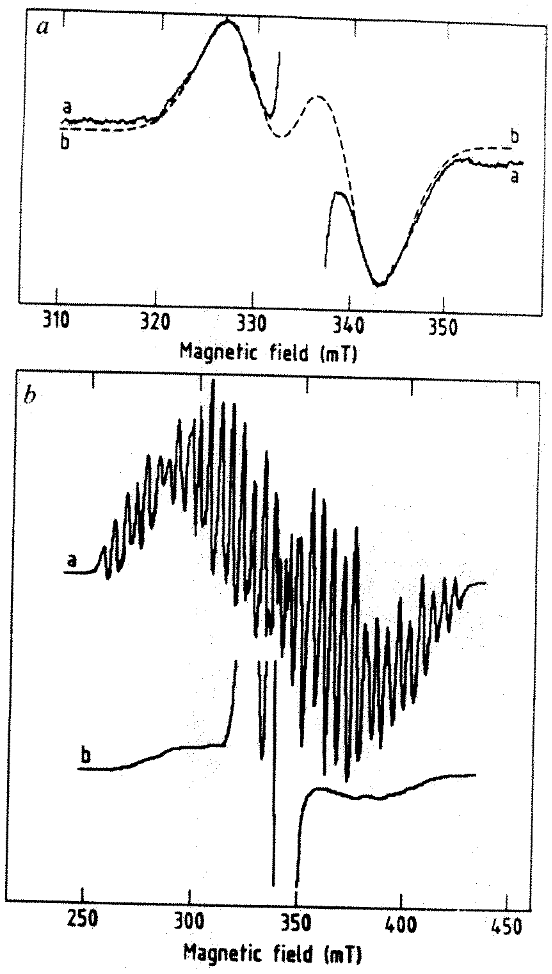

FIG. 2 Simulation of the S_3 EPR spectrum. *a*, Spectrum a shows the experimental S_3 spectrum induced by flash illumination. Spectrum b shows the calculated spectrum for a radical ($S_{rad}=\frac{1}{2}$) interacting with a model system ($S_c=\frac{1}{2}$) that mimics the properties of the Mn cluster in the S_2 state. As the electronic structure of the Mn cluster responsible for the S_2 multiline EPR signal is not known, a simple phenomenological model involving a Mn tetramer ($S_c=\frac{1}{2}$, $I_1=I_2=I_3=I_4=\frac{5}{2}$, $A_1=140.1\times10^{-4}\ \mathrm{cm}^{-1}$, $A_2=70.0\times10^{-4}\ \mathrm{cm}^{-1}$, $A_3=49.3\times10^{-4}\ \mathrm{cm}^{-1}$, $A_4=45.4\times10^{-4}\ \mathrm{cm}^{-1}$ where I_i and A_i are respectively the nuclear spin and isotropic coupling constant of atom *i*) was used here as the $S_c=\frac{1}{2}$ system. This model gives a multiline signal (spectrum a, panel *b*) with an average hyperfine spacing of 55 G, which is similar to that observed for the S_2 multiline signal in the inhibited enzyme. The parameters for the model Mn tetramer are taken from the work of J. Bonvoisin, J.-J. Girerd and J.-L. Zimmermann (manuscript in preparation). The type of magnetic interaction occurring between the free radical and the Mn-cluster may be dipole–dipole and/or electron exchange. It seems unlikely however, that the interaction can be explained in terms of a simple dipolar coupling, as the relaxation properties of the Mn multiline signal, which are rapid relative to those of an organic free radical, would result in only a broadening, rather than a splitting, of the radical signal (for example, refs 8, 22). Thus it is likely that the interaction is dominated by a weak electron exchange. The hamiltonian $\mathcal{H}=g_{rad}\beta S_{rad}\cdot H+g_c\beta S_c\cdot H+(I_1\cdot A_1+I_2\cdot A_2+I_3A_3+I_4\cdot A_4)\cdot S_c+J\cdot S_{rad}\cdot S_c$, with isotropic *g*, *A*, and *J* (*J* is the exchange coupling constant and the other parameters have their usual meanings) tensors was diagonalized and the energy levels and EPR transition probabilities were calculated as in refs 23 and 24. The following simulation parameters were used: $g_{rad}=2.012$, $g_c=1.965$, $J=0.0063\ \mathrm{cm}^{-1}$. Other examples of exchange interactions of this magnitude between organic free radicals and metals have been reported[23,25]. The resulting stick spectrum was convolved with gaussian lineshape functions with $\Delta H_{rad}=43$ G, $\Delta H_c=40$ G to yield spectrum b. These large linewidths for the two interacting spins had to be included to reproduce the shape of the experimental spectrum. Such broadening could arise from some dipolar coupling. A distribution of *J* values over a limited range could also result in such broadened lines[26]. We note that the *g* value of oxidized histidine[27] ($g=2.009$) is similar to that of the free radical involved in the proposed magnetic interaction. *b*, Spectrum a is the calculated EPR spectrum of the hypothetical Mn tetramer which is used as the $S_c=\frac{1}{2}$ system in the simulation of the S_3 signal. Spectrum b is the same simulation of the S_3 signal as shown in panel *a* except that it is plotted on a field and amplitude scale allowing a comparison with the Mn multiline signal. The amplitudes of spectra a and b are scaled relative to the amplitudes of their biological counterparts (compare with Fig. 1*b* for example).

showed that this signal could be induced by continuous illumination at 0 °C and was accompanied by the disappearance of the modified multiline signal[4]. This reaction occurs with a high yield using flash illumination (60% after the first flash and ~100% after four flashes; Fig. 1). The high yield of the signal induced by flash illumination suggests that it is unlikely to be due to a nonspecific photooxidation reaction.

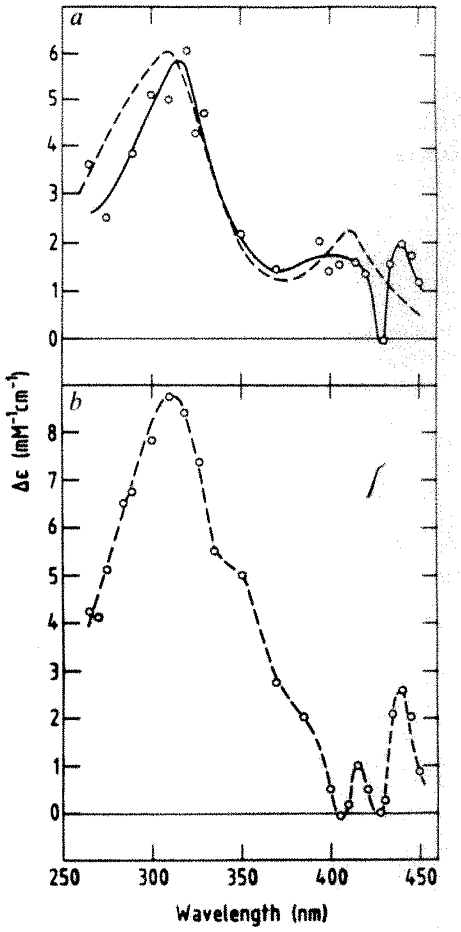

FIG. 3 Absorption changes induced by flash illumination. Absorption changes were measured with the apparatus developed by Joliot *et al.*[28,29]. *a*, Symbols and solid curve: absorption spectrum due to the S_2 to S_3 transition in NaCl-EGTA-treated, polypeptide-reconstituted PS-II membranes. The spectrum was obtained as follows: first, the S_2Q_A to $S_3Q_A^-$ transition was measured in the presence of 10^{-5} M DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) in the 100–250 ms range after four flashes spaced 50 ms apart. Second, the Q_A to Q_A^- spectrum was measured in the presence of DCMU and 2 mM hydroxylamine (an artificial electron donor). Both spectra were normalized to the trough of the C-550 shift caused by Q_A reduction, that is, at 550 nm where no contribution of the donor side occurs[10]. Then the Q_A to Q_A^- spectrum was subtracted from the S_2Q_A to $S_3Q_A^-$ spectrum, resulting in a pure S_2 to S_3 spectrum. Hydroxylamine addition caused about ~20% increase in the Q_A reduction thus measured. This indicates that a fraction of centres could not achieve a stable charge separation. The reproducibility range for the S_2 to S_3 spectrum obtained by this procedure was better than one unit of the ordinate scale. Dashed line spectrum of the oxidized histidine radical at pH 9.2 redrawn from ref. 12. According to these authors, this spectrum corresponded to an $\mathrm{OH}^{\bullet}$ adduct. If the same species occurred *in vivo*, it would represent an intermediate relevant to the water oxidation process. *b*, Spectrum of the S_1 to S_2 transition in untreated PS-II. The same procedure as in *a* was used, except that the DCMU experiment was run by averaging the change recorded in the 100 ms range after each flash in series of 9 (spaced 15 s apart), discarding the data from the first flash. This procedure was used to eliminate any contribution from centres in the S_0 state. The ordinate scale in both panels was calibrated by taking the value of 14 $\mathrm{mM}^{-1}\,\mathrm{cm}^{-1}$ for the peak of the Q_A^- minus Q_A spectrum at 325 mm (ref. 30). The absorption changes were not corrected for the flattening effect. Therefore, the Δε scale is strictly quantitative only around this wavelength. We note that the Q_A^- minus Q_A spectra obtained with treated or untreated PS-II were indistinguishable in the UV region (not shown).

In Fig. 1, the EPR signal induced by flash illumination is better resolved than that reported earlier[4]. It is now evident that the signal is split into two lines, rather than being simply a broad gaussian[4]. These spectral features and the microwave power saturation characteristics[4], indicate that the signal arises from an organic free radical that interacts magnetically with a faster relaxing paramagnet (see for example, ref. 8 and Fig. 2 legend). The loss of the Mn multiline signal, which accompanies the formation of the S_3 signal, indicates that the Mn cluster is the faster relaxing paramagnet that interacts with the organic free radical.

The flash-induced signal can be simulated assuming a weak exchange interaction ($J = 0.0063\ \mathrm{cm}^{-1}$) between an organic free radical and a hypothetical mixed-valence Mn tetramer having spectral properties analagous to the S_2 state (spectrum a, Fig. 2*b*). The simulation is of particular interest as it reproduces not only the asymmetric line shape of the S_3 signal (Fig. 2*a*) but also the collapse of the hyperfine pattern of the Mn signal (spectrum b, Fig. 2*b*). The simulation provides a simple, specific, theoretical model that can explain the EPR data. It shows that the straightforward interpretation of the S_3 signal, as an organic free radical interacting magnetically with the Mn cluster, is physically reasonable.

In our original report on the S_3 signal we did not rule out the possibility that it might arise from Mn oxidation. But this was considered as a rather remote possibility because it was difficult to accept that the new S_3 signal, which is essentially a spin-$\frac{1}{2}$ state, could be formed by a one-electron oxidation of a Mn cluster that already exhibits the spin-$\frac{1}{2}$ state multiline signal; also, it seemed unlikely that such a narrow, featureless signal could arise from a Mn cluster[4]. The new observation that the signal is split around $g = 2$, as well as being indicative of an organic radical, is even less reconcilable with it arising from Mn.

Figure 3*a* (solid curve) shows the near ultraviolet absorption spectrum for the S_2 to S_3 transition in the inhibited enzyme. As found in the EPR experiments, a single saturating flash caused absorption changes that amounted to ~60% of those obtained after four flashes. For comparison, the spectrum of the S_1 to S_2 transition, obtained using a similar procedure with untreated PS-II, is shown in Fig. 3*b* (note that this spectrum is very similar to that of the S_1 to S_2 transition reported previously[9,10]). The spectrum of the S_2 to S_3 transition in the active enzyme of living algae was previously reported to have roughly the same peak position as that of the S_1 to S_2 transition, with an extinction coefficient smaller by about 25%, and a broader bandwidth[10]. The first of these features are exhibited by the S_2 to S_3 transition in the inhibited enzyme (Fig. 3*a*) but the broader bandwidth is not observed. It is possible that the same electron carrier is photo-oxidized in the S_2 to S_3 transition in both the inhibited and native enzyme. The minor spectral differences could be due to structural modifications in the inhibited enzyme and/or to the deconvolution procedures used in earlier work, which were necessary to obtain the S_2 to S_3 spectrum after two photochemical turnovers[10]. An electrochromic bandshift of chlorophyll *a* around 433 nm is also observed in the S_2 to S_3 spectrum (Fig. 3*a*), which is similar to that occurring on the S_1 to S_2 change (Fig. 3*b*), but which was absent in the S_2 to S_3 spectrum from the active enzyme[10]. This suggests that a net charge increase accompanies the S_2 to S_3 oxidation in the modified system and that no, or only partial, deprotonation occurs.

There are several different organic cofactors associated with the enzyme, namely chlorophyll, pheophytin, carotenoid and hydroquinone, any of which could give rise to the free radical. All of these can be ruled out because their well-known spectra are so different from the experimental spectrum. In the absence of any other candidates, amino acids seem to be the only likely alternative. Amino-acid oxidation is not unexpected as tyrosine oxidation has already been demonstrated in this enzyme[5,11] and because the enzyme must generate uniquely oxidizing conditions to oxidize its natural substrate, water. Indeed, the experimental spectrum of the radical (Fig. 3*a*, solid curve) is remarkably similar to that of oxidized histidine reported previously[12] (Fig. 3*a*, dashed line; see also ref. 13). No other oxidized amino acid[14] has an ultraviolet spectrum similar to the experimental spectrum in Fig. 3*a*. We conclude that histidine probably undergoes oxidation in the S_2 to S_3 transition of the inhibited enzyme.

From the model of the PS-II reaction centre proposed by Trebst[15] and the location of the Mn cluster discussed by Babcock *et al.*[11], the most likely candidates as the oxidized histidine are probably those on the lumenal side of the D_1 protein (that is, His 92, His 190, His 332, His 337). Electron spin echo envelope modulation experiments have recently shown nitrogen coupling to the Mn (V. J. DeRose, V. K. Yachandra, A. E. McDermott, R. D. Britt, K. Sauer and M. P. Klein, personal communication). This could reflect histidine binding as a ligand to the Mn as suggested earlier (for example, ref. 16). It is possible that the histidine shown to be oxidized here is a ligand of the Mn and that the proposed exchange coupling reflects a through-bond interaction. But the weakness of the exchange interaction may argue against direct liganding of the histidine to the Mn.

In most recent models it is suggested that Mn is oxidized on each of the four charge-accumulation steps[1,11]. In fact, there is little evidence to support this idea[1]. By contrast, experimental data from NMR[17] and EPR[18] relaxation studies and X-ray absorption spectroscopy[19] were interpreted as indicating that Mn oxidation does not occur on the S_2 to S_3 transition in the functional enzyme. Indeed, the oxidation of an organic free radical (for example, histidine[19]) and its magnetic interaction with the Mn cluster had already been proposed to explain the disappearance of the S_2 multiline signal[18,19]. In addition, it was pointed out earlier[20] that the midpoint potential of histidine (near 1.0 V; ref. 21) is consistent with its involvement as an oxidized intermediate in the functional water-oxidizing enzyme. An S_3 EPR signal could have remained undetected (because of, for example, increased broadening) in the functional enzyme, because of differences in the nature and magnitude of the magnetic interaction compared with those in the inhibited enzyme. Many of the properties of the S_3 signal in the inhibited enzyme reported here and earlier[4] are consistent with those expected for the normal S_3 state. It is thus reasonable to propose that a histidine is oxdized on the S_2 to S_3 transition in the functional enzyme. □

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Three-dimensional structure of ribonuclease H from *E. coli*

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THE three-dimensional structure of RNase H from *Escherichia coli* was determined at 1.8 Å resolution by X-ray crystallography. The enzyme was found to belong to the $\alpha+\beta$ class of structures, consisting of two distinct domains. The structure implies a possible region interacting with a DNA–RNA hybrid. The Mg^{2+}-binding site essential for activity is located near a cluster of four acidic amino acids— one glutamic and three aspartic acid residues. These residues are completely conserved in the homology alignment of sequences of RNase H and reverse transcriptases from retroviruses and retrovirus-like entities[1,2]. The structural motif of β strands around the Mg^{2+}-binding site has similarities to that in DNase I[3–6].

RNase H, which is found in various organisms from prokaryotes to eukaryotes, endonucleolytically degrades RNA moieties of a DNA–RNA hybrid in the presence of Mg^{2+} and produces 5′-phosphates at the hydrolysis sites[7]. Some findings[8–9] suggest that it participates in DNA replication. As deduced from the DNA sequence of the structural gene, the enzyme consists of a single polypeptide chain of 155 amino acids[10]. Furthermore, Johnson *et al.*[1–2] proposed from computer analysis of sequences that reverse transcriptases from retroviruses, including human immunodeficiency virus, may contain a segment homologous with RNase H from *E. coli* downstream of the RNA-directed polymerase domain. Viral RNase H has been suggested to be responsible for degradation of genomic viral RNA in the DNA–RNA hybrid so that minus-strand DNA is available as a template for synthesis of plus-strand DNA[11]. Here we describe the three-dimensional structure of RNase H determined from X-ray diffraction of single crystals, and its functional implication.

Crystals of RNase H were obtained as reported previously[12]. They were grown in the absence of Mg^{2+} which is essential for the enzymatic activity. The crystals belong to the space group $P2_12_12_1$ with unit cell parameters $a = 44.1$ Å, $b = 87.0$ Å, $c = 35.5$ Å and contain one molecule per asymmetric unit. They diffract X-rays beyond 1.8 Å resolution. Conventional multiple isomorphous replacement (MIR) was used for phase determination. The final statistics of structural determination are summarized in Table 1.

The main-chain folding of RNase H is represented in Fig. 1. The molecule has an irregular ellipsoidal shape with overall dimensions 50 Å × 45 Å × 40 Å. On constructing a distance map[13] it was found to be divided into two domains. The principal domain is constituted from four α helices (αI, αII, αIV, αV) and one large β sheet consisting of three antiparallel β strands (βA, βB, βC) at the N terminus, as well as two parallel β strands (βD, βE). The β sheet shows the usual twist commonly found in many other proteins. The minor domain consists of one α helix (αIII) with roughly two turns and a following loop composed of about 10 residues. The joint between αII and αIII, whose axes make a kink at an angle of 35°, is composed of only

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TABLE 1 Structure determination statistics

Data set	Resolution (Å)	No. of independent reflections	No. of crystals	R_{merge} (%)	Average isomorphous difference (%)	Phasing power	R_{Cullis}
CAD4 (for MIR)							
Native	2.5	5,000	1	‡	-	-	-
EMTS	2.5	4,586†	4	5.00	12.3	2.72	0.47
$HgCl_2$ *	2.6	4,325	2	6.13	11.3	3.59	0.41
5-HgCl-uridine (1) *	2.5	4,825	3	6.21	20.5	3.12	0.44
5-HgCl-uridine (2) *	2.5	4,759†	4	5.36	17.3	2.58	0.55
$BaCl_2$	3.5	1,889	1	‡	10.9	0.88	0.81
FAST (for refinements)							
Native	1.8	10,018	2	6.33	—	—	—

Resolution (Å)	14.81	8.70	6.15	4.76	3.88	3.28	2.84	2.50	Total
No. of reflections	33	112	241	411	630	890	1,192	1,491	5,000
Figure of merit	0.97	0.96	0.92	0.88	0.81	0.75	0.63	0.60	0.71

Intensity data to 2.5 Å resolution were collected using a four-circle diffractometer (Enraf-Nonius, CAD4) on a generator with a sealed fine focus Cu tube. For refinement, another intensity data set at 1.8 Å resolution was collected with a FAST area-detector diffractometer on a GX21 rotating anode X-ray generator (Enraf-Nonius). The heavy atom parameters were refined using the program PROTEIN[24]. $R_{merge}=\sum\sum|I_{h,j}-\langle I_h\rangle|/\sum\langle I_h\rangle$; $R_{Cullis}=\sum||F_{PH}\pm F_P|-F_{H(calc)}|/\sum|F_{PH}-F_P|$ (for centric reflections). Phasing power is the ratio of the root mean square (r.m.s.) heavy atom scattering factor amplitude to the r.m.s. lack of closure error. The initial model for RNase H was constructed by use of the interactive computer program FRODO[25] on an Evans and Sutherland PS390 graphics system. The model was refined with the program PROLSQ[26] running on a FACOM VP400E super computer. Occupancies were held fixed at 1.0 and water molecules which refined to B value >50 Å^2 were deleted from the model. The current R factor is 18% on all data from 5.0 to 1.8 Å and 20% on all data from 10 to 1.8 Å after restrained B factor refinement where 84 water molecules are included. The r.m.s. deviations from ideal bond lengths and 1–3 bond angle distances were 0.013 Å and 0.039 Å, respectively.

* Derivatives prepared in the presence of 2-mercaptoethanol. The major site of EMTS, $HgCl_2$ and 5-HgCl-uridine (2) lies near Cys 63. Another 5-HgCl-uridine (1) derivative, which was prepared at a different ratio of 2-mercaptoethanol to 5-HgCl-uridine, has a minor site in addition to the major site.

† Additionally include Bijvoet pairs.

‡ Entire data set collected from a single crystal.

a few residues, not including proline. Data base analysis shows[14] no similar kink in the three-dimensional structures of other proteins. Consequently, the enzyme can be classified into an $\alpha+\beta$ structure[15]. The five-stranded β sheet and a set of two α helices (αII, αIII) form a large cleft with the bottom of αI and αIV in the middle of the molecule. All tryptophan residues at 81, 85, 90, 104, 118 and 120 are concentrated around the cleft.

RNase H requires Mg^{2+} for activity, and the position of Mg^{2+} is assumed to be close to the catalytic site. To identify the Mg^{2+}-binding site, a difference Fourier map $(\{|F_{Mg}|-|F_{free}|\}\exp\{i\alpha_{MIR}\})$ based on MIR phases was calculated between the Mg^{2+}-bound crystal, which was grown in a solution containing Mg^{2+}, and the Mg^{2+}-free crystal. The map indicated a significant peak near the amino-end of αI and the loop connecting βA with βB (Fig. 1*b*). To obtain further evidence, we soaked crystals in a solution of metal ions such as Ba^{2+}, Co^{2+} and Ca^{2+}, whose atomic numbers are greater than that of Mg^{2+}. HPLC analysis of digestion products revealed that Ba^{2+}

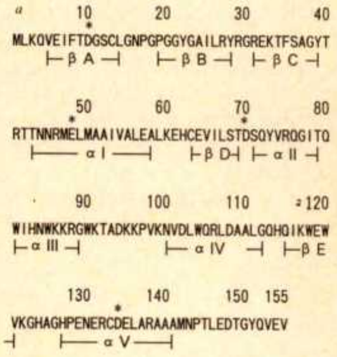
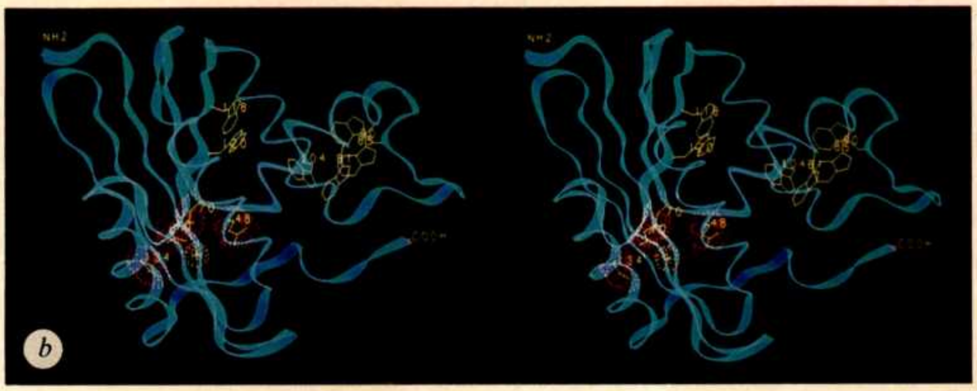

a
10 20 30 40
MLKQVEIFTDGSCLGNPGPGGYGAILRYRGREKTFSAGYT
βA
βB
βC
50 60 70 80
RTTNNRMELMAAIVALEALKEHCEVILSTDSQYVRQGITQ
αI
βD
αII
90 100 110 120
WIHNWKKRGWKTADKKPVKNVDLWQRLDAALGQHQIKWEW
αIII
αIV
βE
130 140 150 155
VKGHAGHPENERCDELARAAAMNPTLEDTGYQVEV
αV

FIG. 1 *a*, Amino-acid sequence and secondary structure of RNase H from *E. coli*. The ranges of five helices and five β strands are shown underneath the sequence. *, the four acidic amino-acid residues, which are conserved completely among sequences in the homology alignment (see Fig. 15 in ref. 2) for retroviral reverse transcriptases, other reverse transcriptase-bearing entities present in the genomes of eukaryotes as well as for RNase H from *E. coli*. The amino acids forming the minor domain are roughly residues 80–100. *b*, Ribbon representation of the main chain folding of RNase H. The minor domain, consisting of αIII and the loop leading to αIV, is located at middle right. All tryptophans, which are indicated by green bonds with residue numbers, are concentrated in a cleft surrounded by αII, αIII, αIV and βE. The Mg^{2+}-binding site essential for the catalytic activity is shown near the bottom left. A yellow sphere indicates the position of Mg^{2+} bound to the site. The three aspartic acid residues and one glutamic acid residue are represented by orange spheres and yellow bonds with residue numbers.



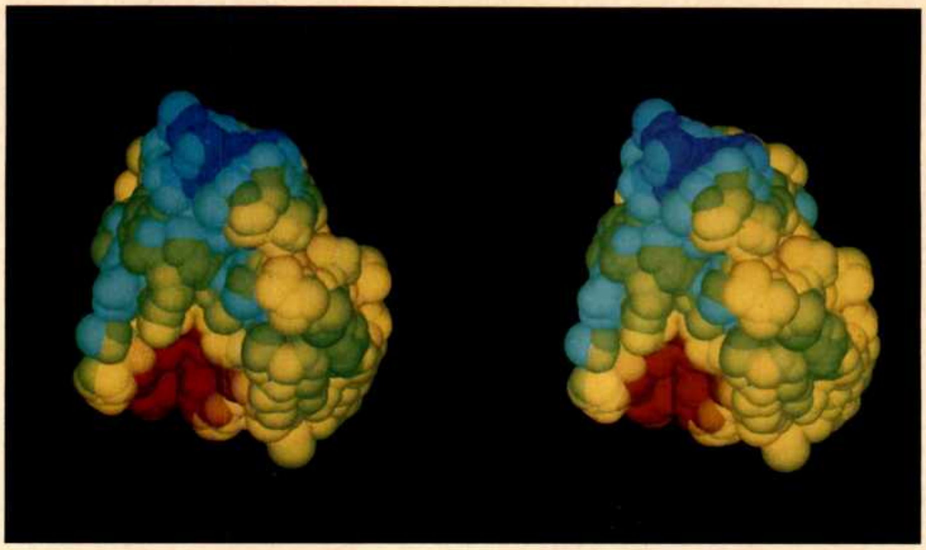


FIG. 2 Distribution of the electrostatic potential[17] on the solvent-accessible surface of RNase H from *E. coli*. Colour codes for electrostatic potential values: red, below −0.1 V and blue, above +0.1 V. Other colour codes correspond to values between 0.1 and −0.1 V sequentially following the spectrum from blue to red. The catalytic activity of RNase H is optimum at pH 8.0, and hence the electrostatic potential was calculated in a condition where histidine residues are not protonated. The red region at the bottom indicates the Mg^{2+}-binding site, and the blue region indicates the basic minor domain. Both regions are supposed to interact with a DNA–RNA hybrid. This interface between the enzyme and the nucleic acid has been supported by recent NMR measurements (Y. Oda and H. Nakamura, unpublished results), in which all signals of the main chain affected by adding a DNA–RNA hybrid oligomer in a RNase H solution were assigned. All residues accompanying large changes in chemical shifts are localized in a region including the Mg^{2+}-binding site, the basic minor domain and residues lying between them.

and Ca^{2+} are less efficient at promoting the digestion of RNA than Mg^{2+} (S. Iwai, M.I., K.M. and E. Ohtsuka, manuscript in preparation). The difference Fourier maps indicated a single, common, significant peak for Ba^{2+} as well as for Ca^{2+}, and the highest peak for Co^{2+} at about the same position as that of Mg^{2+}.

Interestingly, this metal position is surrounded by four acidic residues, suggesting electrostatic interaction between these cations and the negative charges of the carboxyl groups. The distances between the Mg^{2+} position in the derivative (standard deviation of position: 0.16 Å) and carboxyl oxygens in the refined metal-free structure were measured to be roughly 2.0 Å for Asp 10, 4.2 Å for Glu 48, 4.5 A for Asp 70 and 4.9 Å for Asp 134. Some chemical groups, which possibly coordinate to Mg^{2+}, were found around the position, for instance, the carboxyl oxygens of Asp 10 (2.0 Å) and the carbonyl oxygen of Gly 11 (2.3 Å). In the case of Ba^{2+}, two water oxygens (3.4 Å) additionally lie in a possible range of coordination. The Mg^{2+} position was found to be shifted further inward within the metal binding cavity than those of Ba^{2+} or Ca^{2+}. We observed a systematic shift of ionic centres in a reasonable order, according to the ionic radii of Ba^{2+}, Ca^{2+}, Mg^{2+} and Co^{2+}.

The additional chemical groups for coordination would presumably be derived from a polynucleotide main chain during hydrolysis. In the absence of nucleic acid, however, a basic region comprising Lys 86, Lys 87 and Arg 88, which belong to an adjacent molecule, is approaching this empty Mg^{2+}-binding site by electrostatic interaction with the cluster of acidic residues.

We determined the three-dimensional structures of RNase H mutants whose Glu 48 and Asp 70 were replaced by Gln and Asn respectively K.K., I. Tanaka, S.K., M.I. and K.M., unpublished results). Their structures showed movements of some side chains and formation of extra hydrogen bonds around the Mg^{2+} site which seem to prevent the binding of Mg^{2+}. These conformational changes would account for the deprivation of the activity.

Recent measurements of RNase H by ^{1}H and ^{15}N NMR revealed that the chemical shifts of particular residues around the Mg^{2+}-binding site, such as Asp 10, Gly 11, Asp 70 and Gln 72, were affected by addition of 50 mM Mg^{2+} (Y. Oda and H. Nakamura, unpublished results), supporting the correct identification of the Mg^{2+}-binding site.

The proposed location of Mg^{2+} is consistent with the results of a series of site-directed mutagenesis experiments in which amino-acid residues at various positions were replaced with others (data not shown). The enzymatic activity was almost completely lost by replacement of Asp 10 by Ala or Asn, Glu 48 by Ala or Gln and Asp 70 by Ala or Asn, but not by the replacement of Asp 134 by Asn which is the most distant from Mg^{2+} (4.9 Å). The loss of activity can be explained by disruption of the Mg^{2+}-binding site, although some of the three acidic residues possibly participate directly in hydrolysis of RNA.

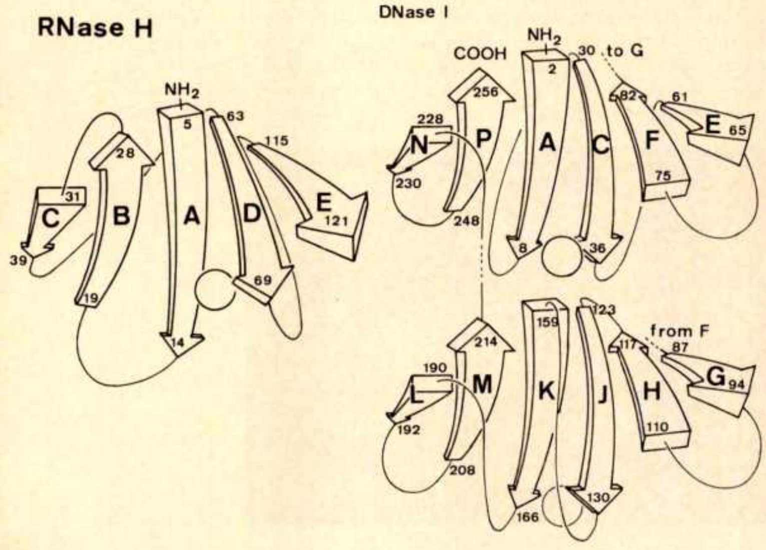


FIG. 3 Schematic representation comparing the directional relationship of β strand topologies between *E. coli* RNase H and DNase I. Circles indicate the Mg^{2+} position for RNase H and the Ca^{2+} position for DNase I, respectively. DNase I (ref. 6) has two β sheets related by an approximate two fold axis but a single catalytic Ca^{2+}, and hence two circles indicate the identical position. The directions and the twist of four β strands (CBAD) in RNase H are identical to those of two β sheets of DNase I (NPAC and LMKJ). In the assumed complex, a DNA–RNA hybrid lies at the end of the β sheet and its axis is roughly normal to the plane. DNase I was reported to bind a double helical DNA in a similar manner[4,5].

The four acidic residues around the Mg^{2+}-binding site, Asp 10, Glu 48, Asp 70 and Asp 134, have recently been reported to be completely conserved in the alignment of 26 different assorted sequences from retroviruses, retrovirus-like entities and RNase H from *E. coli*[2]. This conservation can now be explained by the preservation of the Mg^{2+}-binding site, which is essential for activity.

RNase H does not contain the DNA-binding motif consisting of helix-turn-helix, which can commonly be found in repressors[16]. The minor domain, αIII and the following loop, contains many basic residues (Fig. 1*a*) whose side chains mostly point into the solvent region. This feature implies that the minor domain is involved in binding to a DNA-RNA hybrid. It is reasonable to assume that a straight DNA-RNA hybrid double helix would bind to the enzyme, along the line linking the acidic Mg^{2+}-binding site with the basic minor domain. A molecular graphics display of the electrostatic potentials[17] on the solvent-accessible surface indicates the cluster of negative charges around the Mg^{2+}-binding site and the minor domain covered with positive charges (Fig. 2).

We could recognize no significant similarity to the three-dimensional structures of nucleases such as RNase A[18-19], RNase T$_1$[20-21], barnase[22] or staphylococcal nuclease[23]. With respect to specificity and substrate preference, RNase H seems to be similar to pancreatic DNase I. Both enzymes act on double-stranded polynucleotides without much specificity for base sequence, and both also require metal cations for catalytic reactions (Ca^{2+} for DNase I).

The similarity emerges from the comparison of the β sheets between RNase H and DNase I[3-6] (Fig. 3). The Mg^{2+}-binding site is located near the amino end of βB and the carboxyl ends of βA and βD. Suck and colleagues reported the identification of the amino-acid residues involved in the catalytic reaction and DNA recognition in DNase I[5-6]. The catalytic Ca^{2+}-binding site is located at the amino end of βP, at the carboxy ends of βA and βC in the first β sheet, and at the amino end of βM, as well as at the carboxyl ends of βK and βJ in the second β sheet. Interestingly, the directional relationships of βC to βD and the twist of β sheet in RNase H are the same as those of the corresponding β strands in two β sheets of DNase I. In both enzymes, the numbers of residues forming β strands range mostly from 7 to 10.

Note added in proof: We are still continuing the crystallographic refinement, extending the intensity data to 1.48 Å resolution. The current *R*-factor is 20.9% on all data from 5.0 to 1.48 Å, and the electron density map has been considerably improved.

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Advances in automated DNA sequencing

R. Cathcart

Sequencing DNA is a labour intensive and expensive process. At an estimated cost of \$3–5 (US) per base pair, it would require about \$25 million (US) to complete the sequence of *Escherichia coli*[1].

THIS article looks at the progress in automated DNA sequencing to increase throughput and reliability. New hardware and improvements in sequencing chemistry developed by Applied Biosystems (ABI) are discussed.

Hardware developments

The ABI Model 373A automated DNA sequencing system can run 24 templates simultaneously. One of the rate-limiting steps now becomes performing the sequencing reactions. The risk of pipetting errors and sample mix-ups can increase with this number of samples. A robotic workstation is under development at ABI that is designed to perform sequencing reactions on up to 24 different templates simultaneously (see Fig. 1).

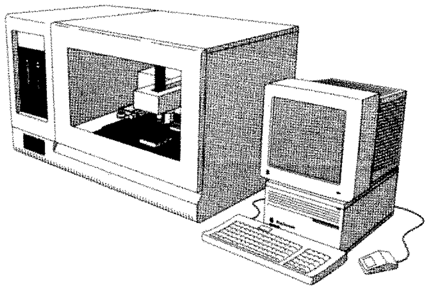

FIG. 1 DNA sequencing robotic workstation.

The worksurface contains refrigerated areas for storing labile reagents such as enzymes and templates, as well as an area for ambient storage. Reagents are pipetted from their storage area to the enzyme reaction module (ERM) using a syringe-based system coupled to a fixed stainless steel probe tip at the end of the robotic arm (see Fig.2).

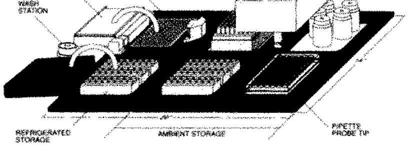

FIG. 2 Details of the worksurface.

An important design criterion of the ERM is evaporation control. This requirement is dictated by the high temperature (95 °C) and small volumes (5 μl) of *Taq* DNA polymerase-based sequencing protocols. An oil 'cap' is effective in preventing evaporation in manual methods, but is not suitable in automated systems. Instead, the ERM has a lid that automatically closes to seal the enzyme reactions. Wells are machined into an aluminium plate, which is then coated with an inert polymer. The well size is matched to the volume of the reaction to minimize condensation. A gasket in the lid seals all 96 wells by compressing on a machined rim. To prevent condensation from occurring on the lid while in the closed position, it is heated to 2 °C above the reaction temperature.

The aluminium plate is a thermal cycler with a temperature range from 2 to 95 °C. The high thermal conductivity of the aluminium ensures rapid and uniform heating or cooling of the samples. To initiate all reactions simultaneously, the robot loads the ERM at 4 °C and then rapidly heats the plate to the desired temperature. This design prevents evaporation and ensures temperature uniformity between wells. The obvious disadvantage of a reusable plate is that it must be cleaned. However, a relatively simple and rapid method for purging the block between reactions has been determined.

Reagents are pipetted using a single stainless steel probe tip coupled to the syringe pumps. A positive displacement effect is achieved by filling the pumps and line with water. The system can detect when the probe tip touches a liquid surface because the stainless steel probe tip has capacitive sensing. This allows reagents to be delivered at the surface of the reaction, which minimizes probe tip contamination. Increased reliability and lower cost are also achieved with this design because disposable tips do not have to be coupled and uncoupled from the system.

The most important design criterion, however, is the ability to pipette submicrolitre quantities. Prototype instruments are able to pipette 1 μl and 0.1 μl with a variation of one and eight per cent, respectively. This level of performance was impossible with disposable tip systems. Again, the disadvantage is the probe tip must be washed between pipetting steps.

Prototype instruments are able to perform fluorescent primer sequencing reactions using T7 or *Taq* DNA polymerase on 24 samples in less than 4.5 hours. Less than one hour is required to clean the ERM between runs. One robot can easily supply the needs of two automated sequencers, such as ABI's 373A.

With sequencing protocols, contamination between samples is not a problem. The normal ERM and probe tip washing protocols are effective at cleaning the system. The obvious concern is for polymerase chain reaction (PCR) applications. Recent experiments suggest that both the wells and the probe tip can be cleaned of amplified DNA by use of dilute nitric acid. Further work is needed in this area, but it seems likely that a cleaning protocol suitable for PCR applications can be devised.

Chemistry improvements

Although the robotic workstation reduces labour requirements and error, and increases the reliability of sequencing, not all applications require this level of throughput. Improvements in the *Taq* sequencing protocol have reduced both the labour requirement and cost of performing sequencing reactions manually. This has been achieved with linear amplification of signal by thermal cycling with a single fluorescent primer (in contrast to the exponential amplification achieved with PCR). Both labour and error are reduced for manual sequencing because the separate annealing step is no longer needed and a denaturation step is not required with double-stranded templates. Costs are reduced because less enzyme and template are required. The need for less template permits the use of larger inserts for primer-directed sequencing, such as cosmid sequencing in which the mole per cent of real target is considerably less than with M13 and plasmids (R. Kaiser, personal communication). Fluorescent signal level remains high because of the linear amplification. The combination of the robot and the *Taq* cycle sequencing protocol should allow further reductions in the amounts of reagents required. ☐

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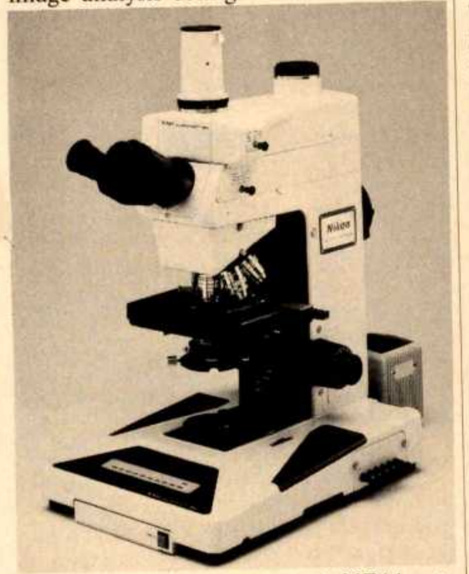
Lab Week product profile

New lines in laboratory hardware, including a video image recorder and a low-volume evaporator, will be on display at next week's British Laboratory Week exhibition to be held in London.

K.W. Kirk will be displaying an **epi-fluorescence illumination unit** for field and laboratory use or on-the-spot diagnoses on stand D1446 at British Lab Week (*Reader Service No. 101*). The compact, modified standard McArthur microscope measures 7.6 × 17.8 × 15.2 cm and uses epi-illumination of the specimen to provide a high level of illumination while protecting the user from the harmful effects of UV, says Kirk. The £2,980 (UK) McArthur fluorescence equipment provides magnifications of 100×, 400× and 1,000× and is useful where another fluorescence microscope system would either be too costly or impractical.

The Microphot-SA from Nikon is a **microscope designed for image analysis** applications where CCTV observations and photometry are more important to the researcher than photomicrography alone (*Reader Service No. 102*). The microscope is equipped with two output ports: one for a CCTV camera and the other for photomicrography. The permanent CCTV image analysis configuration is achieved by the use of two independent three-way beam splitters, which provide all possible combinations of observation, CCTV and photography. The Microphot-SA can be linked to a PC via a RS232 interface for either remote control or recording purposes. Key automatic features of the microscope include auto-focus, a motorised nosepiece and condenser, and pre-centred lamphouse. See Nikon on stand K1202.

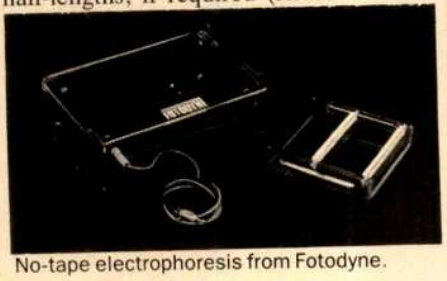

Microphot-SA: for a permanent CCTV image analysis set-up.

The FreezeFrame **video image recorder** from Polaroid can prepare 35-mm colour or black and white slides and prints from screen images (*Reader Service No. 103*). FreezeFrame is PAL-compatible and accepts RGB analogue and RGB/TTL computer input signals. Consequently, images can be taken from a VCR, VTR, laser disk player or straight from a video camera and recorded on instant photographic prints or 35-mm slides for use in lectures, file records or reports. The instrument 'grabs' a video field from the 'live' image, which can then be adjusted for colour, contrast and brightness before it is captured on film. When used with Polaroid's instant film power processor, FreezeFrame can perform on-the-spot processing of Polaroid's 35-mm full tonal range black and white PolaPan slides, normal-contrast PolaChrome or high-contrast PolaChrome colour slides (recommended for computer image recording). FreezeFrame can also record images on Polaroid's AutoFilm. The motorised Polaroid 'back', which is powered by the host equipment, can produce self-developing instant black and white or colour hard-copy prints in a 3 × 4-inch format. Polaroid's products will be on show on stand K1200.

FreezeFrame for slide and print production from video images.

Focusing on electrophoresis

Fotodyne has a new **no-tape electrophoresis chamber** — the Model 1214 — for running 12 × 14-cm agarose gels or half-lengths, if required (*Reader Service*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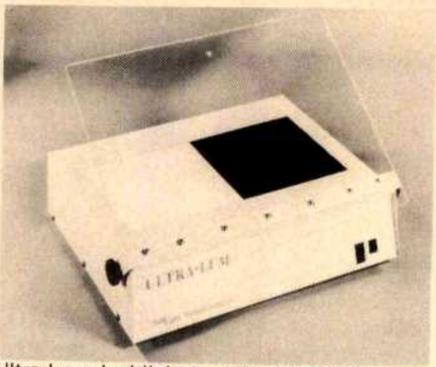
No. 104). The Model 1214's UV-transparent tray is designed to allow gel casting to be performed in the chamber, with no need for taping. By placing the tray sideways in the chamber, end gaskets create a tight, leak-proof seal for gel formation, says Fotodyne. After the gel has set, the tray is then returned to its normal position. The electrophoresis chamber is supplied with partially shielded platinum electrodes, 12- and 20-tooth combs, power leads attached to the lid, buffer exchange ports, and a buffer capacity of 500 ml. The chamber's design also features an evaporation gap to minimize the problem of condensation within the unit, and non-skid rubber feet to provide stability. The Model 1214 costs $225 (US).

The Chef Mapper **pulsed field gel electrophoresis system** from Bio-Rad sets out to eliminate some of the trial and error associated with optimizing pulsed field separations (*Reader Service No. 105*). Parameters for switch time, pulse angle, switch time ramp, voltage, run time, agarose type and concentration, buffer type and concentration, and temperature are set automatically on the basis of stored protocol information and entered details of DNA size. More experienced users can bypass the algorithm that controls the automation process. For example, changing the pulse angle from 120° to 96° can, says Bio-Rad, cut run times for megabase DNAs in half. Other key features of the Chef Mapper system include multi-state programming of up to 15 vectors per pulse cycle, linear and non-linear switch time ramps, and secondary pulses. H136 is the Bio-Rad stand.

The **megabase restriction mapping system**, which comes in kit form from New England BioLabs, contains all the reagents necessary to resolve a wide range of restriction fragments by pulsed field gel electrophoresis of chromosomal DNA (*Reader Service No. 106*). Each $125 (US) kit contains restriction endonuclease for digestion of agarose-embedded chromosomal DNA, 10× reaction buffer, control bacterial chromosomal DNA substrate, and lambda ladder and yeast chromosome pulsed field gel markers. Also included in the kit is the GelSyringe system for dispensing agarose plugs of consistent size and quality, says NEB.

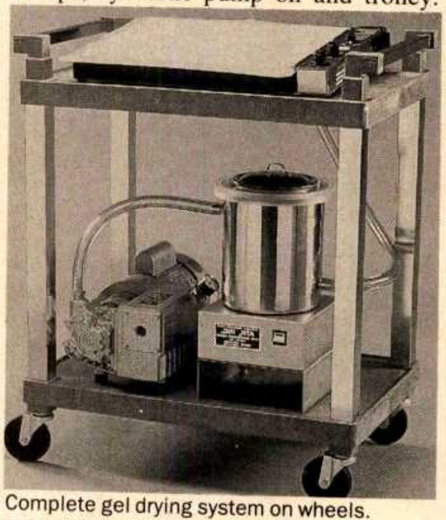
Molecular marketplace

The Ultra-Lum **dual-light transilluminator**, distributed by VWR Scientific, features two light sources in one instrument: a UV light source and a 'cool' fluorescently lit working surface (*Reader Service No. 107*). Two models are available: the $1,295 (US) UVBW-20, which features four 8-W, 300-nm UV tubes and provides the sensitivity required for viewing ethidium bromide-stained gels while preventing photodamage, photobleaching, photonicking and photodimerization, says VWR; and the $1,095 (US) UVAW-20, which has four 8-W, 365-nm UV tubes. Both transilluminators also feature two 8-W cool-white fluorescent tubes for viewing silver- and Coomassie blue-stained protein gels and methylene blue-stained nucleic acid gels, autoradiographs and microtitre plates.



Ultra-Lum dual-light transilluminator features a fluorescently lit workstation.

Key features of the new GDS-1 **gel drying system** from ATR/AHL are large gel dryer slab capacity, variable temperature control, acid-resistant design, and an automatic vent/drain mechanism (*Reader Service No. 108*). The $2,725 (US) system comes complete with gel dryer, pump, automated dry ice trap, vacuum tubing, clamps, synthetic pump oil and trolley. The 36 × 44-cm drying surface can accommodate six 14 × 16-cm slab gels or three 14 × 32-cm slabs. Gel drying temperatures can be adjusted over the range of 40 °C to 80 °C. The dryer features two timers: one to control the vacuum pump, the other to operate the heating circuit. Both dry heat and reduced pressure are used to dehydrate slab gels and bond them permanently either to filter paper or transparent porous cellophane. The automated vacuum condensing trap is designed to both protect the vacuum pump in corrosive environments and offer a means of collecting and discarding unwanted solvents.

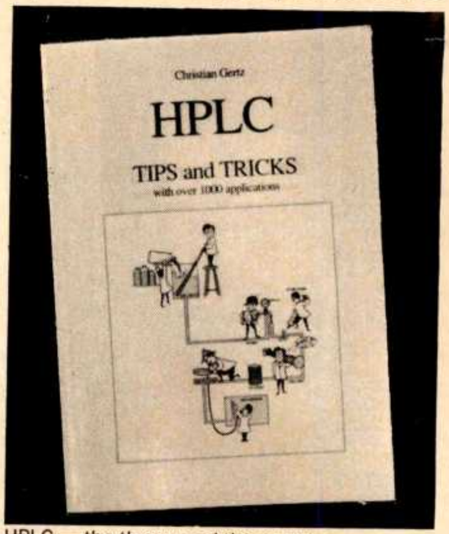


Complete gel drying system on wheels.

The **miniblotter incubation system** from Biometra is designed to provide a rapid method for screening antibodies on western blots by eliminating the need to cut blot membranes (*Reader Service No. 109*). Up to 45 antibodies can be screened on a single blot, without problems of cross-channel leakage, says Biometra. The screening procedure requires only small volumes of antibody solution — typically 50–250 μl. The £495 (UK) miniblotter incubation system has a detachable manifold to allow thorough washing of the blot.

Chromatography corner

Tips and Tricks, a new **HPLC textbook** from LDC Analytical, covers all aspects of HPLC practice and should be of interest to novices and experts alike (*Reader Service No. 110*). This 500-page publication covers the theory of chromatographic separation and contains practical advice on how to select the most suitable stationary and mobile phase, how to check the reproducibility of your chromatographic system, how to build, operate and maintain an HPLC system, and how to evaluate and quantitate results. The £35 (UK) *Tips and Tricks* book contains 1,000 up-to-date references and will be available on the LDC stand, number K1016.



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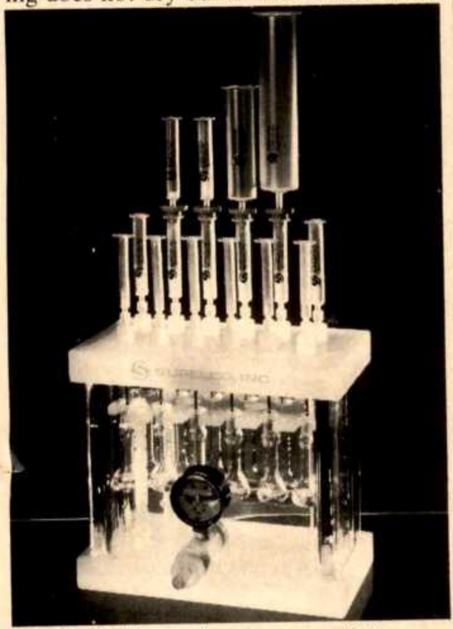
HPLC — the theory and the practice.

Polymer Laboratories has brought out a new matched series of PL-GFC **macroporous polymeric resins** with a hydrophilic surface for gel filtration chromatography (*Reader Service No. 111*). The resins can be used for the analysis of a variety of water-soluble macromolecules, including charged and neutral polysaccharides. PL-GFC resins are available in three pore sizes — 300 Å, 1,000 Å and 4,000 Å — for use individually or, if increased resolution or resolution over a broader molecular size range is required, in multi-column sets. The 300 Å and 4,000 Å materials have matched pore sizes to allow them to be used in series to cover polysaccharide molecular weights ranging from several hundred to about 20 million, says PL. The hydrophilic homogeneous surface and chemical stability of the polymer matrix permits the use of a wide variety of aqueous eluents; this reduces non-specific interactions and enables polysaccharides to be analysed in the presence of proteins, without the problem of irreversible column fouling. See PL Separation Sciences on stand K1328.

Spectra-Physics has introduced the SP8880B **biocompatible autosampler** which is designed for applications where wetted parts made of metal would interfere with metal-catalysed reactions in the sample (*Reader Service No. 112*). All the wetted parts in the SP8880B are made of PEEK (polyetheretherketone) instead of titanium. The company has also replaced the Vespel rotor seal in the rheodyne 7010 valve with one made of Tefzel, which tolerates a wider pH range, says Spectra-Physics. The autosampler holds up to 80 samples and can perform ten injections per sample. When the SP8880B is teamed up with an inert pump and a detector that has a biocompatible flow cell, users can configure a complete biocompatible LC system that can also be integrated into Spectra-Physics' communications network — Labnet. Prices start at $17,000 (US).

Using the Supelco Visiprep **solid-phase extraction vacuum manifold**, it is possible to clean up to 12 samples simultaneously for HPLC or other analyses (*Reader Service No. 113*). The apparatus has twelve independently-controlled screw-type valves built into the cover, which provide precise flow control through each SPE tube. The vacuum is adjusted using the vacuum bleed valve and guage. Such control ensures, says Supelco, that the packing does not dry out in some tubes while others are still draining. The £540 (UK) SPE vacuum manifold has a rugged, clear-glass basin that provides clear visibility during the extraction process and will not fog or discolour even when exposed to solvents such as hydrochloric acid and tetrahydrofuran. The polypropylene collection-vessel rack holds autosampler and small scintillation vials, 10- and 16-mm test tubes, and 1-, 2-, 5- and 10-ml volumetric flasks. By adding an optional plate, samples can be collected in up to ten large (20-ml) scintillation vials. The stainless steel guide needles can easily be removed for cleaning or replacement. Alternatively, where sample-to-metal contact must be avoided, Supelco can provide Teflon solvent guide needles.



Supelco's 12-sample vacuum manifold.

Tools of the trade

The new combination **hotplate/stirrer** from Fisher Scientific uses infrared light to provide temperatures of up to 615 °C (*Reader Service No. 114*). The hotplate has a rapid thermal response time and will boil one litre of water in nine minutes, says Fisher. Two models are available: the smaller $795 (US) Model 4100 with a six-inch diameter heating circle (28 in^2) and the larger $850 (US) eight-inch Model 6100 version (50 in^2). Stirring speeds can be set to between 60 and 1,500 r.p.m. Infrared produced by the molybdenum disilicide element housed beneath the black, glass ceramic top plate glows orange when the hotplate is in use. For safety purposes, the hotplate has a stainless steel lip for catching spills.

Neslab Instruments, exhibiting on stand C512, has introduced a new −80 °C **personal freezer** — the PF8-80 — for the safe and secure storage of radioisotopes and biological samples (*Reader Service No. 115*). Both the −80 °C model and the earlier −40 °C model are fitted with an audio/visual alarm. There is also provision for a remote alarm and temperature monitoring. High security is the freezer's key feature, which is achieved by placing the set point and alarm controls inside the door jamb. Other features include a lockable door and large LED. Neslab's freezer sells for £2,100 (UK).

TurboVap LV, the automated **low-volume evaporator** from Zymark, is designed to evaporate to dryness 1–50 samples simultaneously with volumes from 1–30 ml (*Reader Service No. 116*). The $4,900 (US) evaporator uses nitrogen gas to create a vortex action in the sample and can, says Zymark, evaporate 10 ml of MeCl$_2$ in less than 15 minutes at 38 °C or 10 ml hexane in less than nine minutes at 52 °C. The compact, TurboVap LV uses standard 16 × 100-mm or 20 × 150-mm tubes. The temperature of the bath can be varied from between ambient and 90 °C. Interfacing the TurboVap LV to Zymark's BenchMate workstation provides automated sample preparation and evaporation. See stand A448.

On show for the first time at British Lab Week on Grant Instrument's stand, number H150, will be a **portable incubator** that provides a useful alternative to conventional CO$_2$ incubators (*Reader Service No. 117*). Because of the incubator's portable design, samples can be kept to hand while the user is working at the bench where space is often limited. The incubator can provide a stable temperature of either 37 °C or 56 °C and an internal atmosphere of constant humidity and five per cent CO$_2$ in air. After initial gassing with a bottled gas mixture, conditions are maintained for up to 48 hours, says Grant. The incubator can accommodate test tubes, petri dishes or a combination of both. It can also be converted to a standard heating block or stand-alone base plate.

Portable incubator — see stand H150.

Quantitative analysis

Pharmacia LKB Biochrom will use Lab Week to launch a new Ultrospec III **UV/visible spectrophotometer** with Microsoft Windows PC-based applications software — see stand G182 (*Reader Service No. 118*). Ultrospec III has a spacious sample compartment and can be equipped with a wide range of accessories, including the Autofill sipper rapid sampler, thermostatted cell holders and funnel flow cells, all of which allow the instrument to be adapted to suit laboratory needs. The new spectro colour software, operating in a Microsoft Windows environment with mouse control if possible, can be used with IBM PCs or compatibles. The software has three modules: enzyme kinetics for enzyme

A Lab Week launch for Pharmacia's UV/visible spec and colour software.

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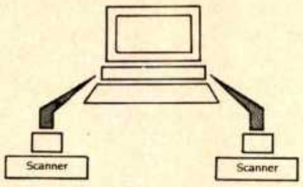
catalysing reaction studies, wavescan for wavelength scanning, and standard curve for quantitative and colorimetric analyses. Pharmacia's 20-page product brochure describes the spectrophotometer in detail.

Molecular Dynamics now offers researchers a choice of three **computing densitometers** (*Reader Service No. 119*). Two new instruments — the Model 300E and 300S — have been added to the product line to provide faster data analysis for gels, films and membranes. The Model 300S, which is fitted with a 16 MHz Intel 80386SX microprocessor, can display and quantitate image data up to 60 per cent faster than the 300A, says Molecular Dynamics. All three models are designed to scan electrophoresis gels, radiographic films and blotting membranes as large as 36 × 42 cm in less than three minutes. All three use the same helium-neon laser, light-collection cylinder and Image-Quant software, which eases the user through the process of data collection and analysis. See stand G629.

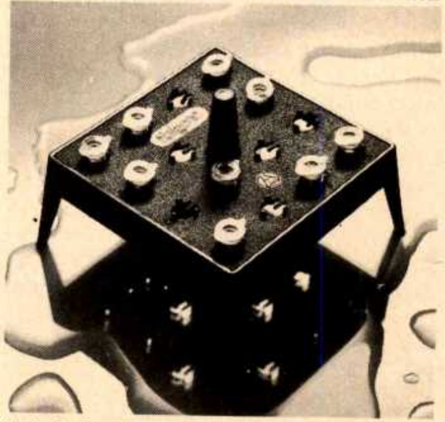
ScanDuet, the automated **TLC radioimaging and analysis system** from Bioscan is designed for high-throughput TLC applications (*Reader Service No. 120*). The instrument, which consists of two automated scanners linked up to a multi-tasking computer, can load and read up to eight 20 × 20-cm plates without user intervention. Users have a choice of 1- or 2-D detection. ScanDuet's detectors can measure 50 d.p.m. of ^{3}H or ^{14}C in ten minutes, says Bioscan. With low-activity samples, 2-D images can be produced in a day and not weeks as with autoradiography. ScanDuet's windowless detector and the use of uncovered plates contribute to the instrument's high sensitivity. To suit user needs, both lane width and resolution can be altered by changing snap-on collimators. The TLC-specific software that accompanies ScanDuet allows the user to display and quantitate data in 1-, 2- and 3-D. Features of the software include automatic peak search, a fine-tuning capability for regions of interest, and computation of peak location, total counts, c.p.m. and per cent of total activity.



High throughput TLC image analysis.

Lab consumables

Throw away your polystyrene tube holders, Nalge is offering a **floating microtube rack** for 0.5-ml Eppendorf tubes (*Reader Service No. 121*). The rack holds sixteen 0.5-ml tubes in a 4 × 4 array, allowing incubation of samples at elevated or reduced temperatures in water or ice baths. Made of polypropylene, the rack offers, says Nalge, temperature resistance from −70 °C to 100 °C. The rack comes with moulded-in handles to assist removal from a water bath. Nalge is on stand K1074.



Nalge's floating microtube rack.

Hepa-Vent, the lightweight **in-line microfiltration device** from Whatman can be used in sterile air or gas venting applications (*Reader Service No. 122*). Each filtration device features a polypropylene housing with stepped hose barbed connections and a glass microfibre filter medium that is laminated on both sides and slightly hydrophobic. The device, which is autoclavable, is designed to retain 99.97 per cent of airborne particles that are ≥3 μm. Hepa-Vent's disc format provides 16 cm^2 of filtration area: for larger air flows, however, Hepa-Vent is available with a filtration area of 540 or 1,260 cm^2. See stand G164.

Tissue Culture Services, exhibiting on stand L1192, is offering EquiCell **donor equine serum** as an alternative to fetal calf serum (*Reader Service No. 123*). EquiCell is produced from a controlled herd of donor horses that are screened by BioResearch — Ireland's National Cell and Tissue Culture Centre in Dublin. The serum is suitable for the growth of hybridomas and myelomas, epithelial cell lines, fibroblasts and recombinant cell lines such as CHO and BHK. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

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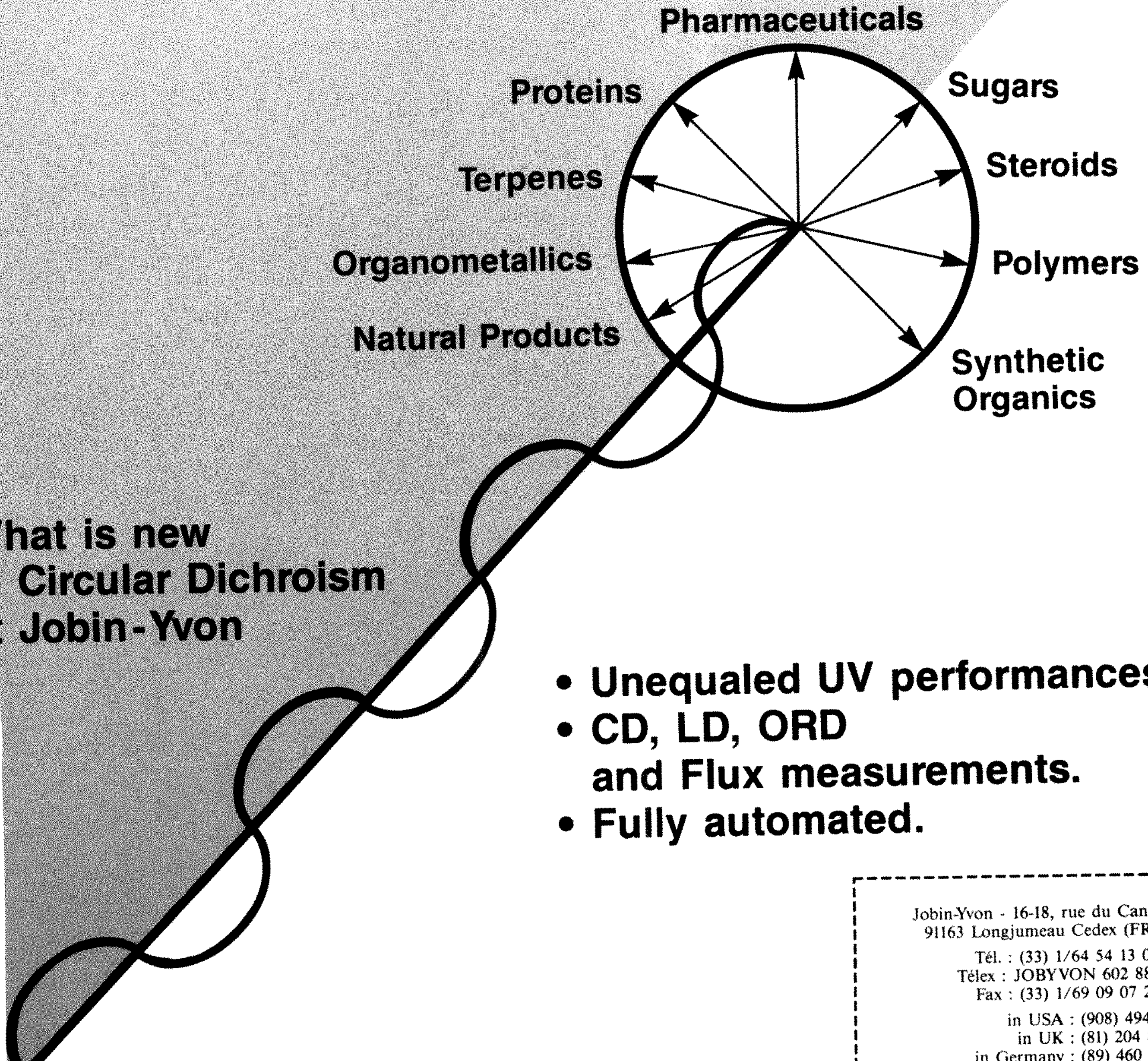

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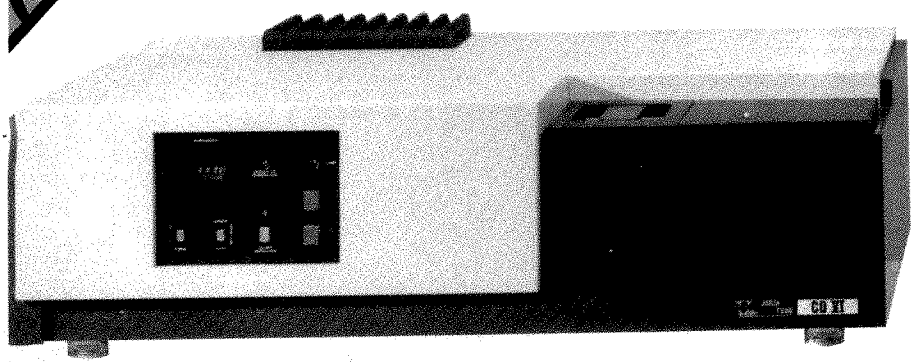

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PARIS Clare Newell, BP No 804, 75828 Paris, Cedex 17. Tel: (1) 48 87 24 43. Fax: (1) 48 87 46 71.

MUNICH – Sabine Fürst, Sandstrasse 41 8000 München 2. Telephone: (089) 52 40 82. Fax: (089) 5 23 22 22.
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JOHN INNES CENTRE FOR PLANT SCIENCE RESEARCH

SCIENTIFIC OFFICER

Required to undertake research into the molecular biology, genetics and biochemistry of particular pea seed proteins. The person appointed will work with a senior scientist in analyzing these proteins, in isolating and characterising their RNA and DNA and in cloning and sequencing the DNA. The position offers opportunities of interacting with a group of scientists working on various aspects of pea molecular biology. Further details can be obtained from Dr Rod Casey.

Candidates should have a degree in an appropriate subject with a particular interest in molecular biology and/or biochemistry.

The salary is £9,907 to £13,262; the post is a straight-through position with promotion to the Higher Scientific grade (salary £11,586 to £13,262) on achieving the appropriate level of experience and expertise. Non-contributory superannuation scheme. Equal opportunities employer.

Applications with full CV and the names of two referees should be sent to the Personnel Officer, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich, NR4 7UH, quoting reference no. AG/488. Closing date for applications is 11th October, 1990. (4642)A

Ontario Cancer Treatment and Research Foundation

Northeastern Ontario Regional Cancer Centre
Sudbury, Ontario

CANCER RESEARCH SCIENTIST

Applicants are invited for a staff career scientist position as a member of an experimental oncology group with a focus on applied clinical research. The successful candidate should have demonstrated excellence in carrying out research in immunology at the cellular and molecular level as a member of a collaborative research group. Considerable experience at the post-doctoral level is required. After a start-up period, on-going support for research will be from competitive granting agencies. Academic appointment will be at the University of Ottawa, and Laurentian University, Sudbury.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada, although others are encouraged to apply.

Closing date for this competition is 11th October, 1990.

Applicants should send resume and names of three referees to:

Anthony D Ho, MD
Director of Research
Northeastern Ontario Oncology Program
41 Ramsey Lake Road
Sudbury, Ontario
Canada P3E 5J1

(NW5800)A

BRUNEL UNIVERSITY and ROYAL HOLLOWAY AND BEDFORD NEW COLLEGE

POSTDOCTORAL RESEARCH ASSISTANT

Required to work on a project to derive novel therapeutic agents which block the binding of HIV to lymphocytes. This project is currently supported as a 12 month pilot study by the MRC AIDS Directed Programme. The experimental programme avoids the use of live HIV and infected human lymphocytes.

Informal enquiries may be made to the joint supervisors Dr D Coombe, Department of Biology and Biochemistry, Brunel (0895 74000 ext 2405) and Dr C Rider, Department of Biochemistry, RHBNC (0784 443548). Applicants should possess or be expecting to gain shortly a PhD in Biochemistry, Cell Biology or Immunology, preferably with some background in carbohydrate analysis. Salary on the scale £13,166-£15,262 including London Allowance.

Application forms are available from the Personnel Office, Royal Holloway and Bedford New College, Egham Hill, Egham, Surrey TW20 0EX. Tel: 0784 443030 and completed application forms should be returned as soon as possible. (4676)A

POSTDOCTORAL POSITION IN MOLECULAR IMMUNOLOGY

One position is available for the study of regulation of immunoglobulin synthesis in human primary immunodeficiency. The department is a major referral center for patients with various forms of immunodeficiency. Research is mainly focussed on aspects of immunoglobulin deficiency. For update on current work see our article in this issue of Nature. Work includes mapping and sequencing of immunoglobulin genes and gene deletion breakpoints. Send c.v. and names of references to **C.I.E. Smith & L. Hammarström, Center for BioTechnology, Karolinska Institute, Novum, S-141 52 Huddinge, Sweden. Tel. Sweden-8-7795204. Fax: 8-7466869.** (4692)A

JOHN INNES INSTITUTE

Department of Genetics: Molecular Biologist

Applications are invited for a Scientific Officer to work in a group investigating the molecular genetics of antibiotic biosynthesis in Streptomyces as part of an on-going international collaboration. Candidates should have a degree or equivalent in a molecular biological/biochemical discipline with, ideally, some experience of gene cloning. This is a temporary post and will continue until the funding body withdraws its financial support.

Salary will be on the Scientific Officer scale: £9,907-£13,262 per annum. Non Contributory Superannuation. Further particulars may be obtained from Professor Hopwood, FRS, John Innes Centre for Plant Science Research, Norwich NR4 7UH. Applications quoting Ref GEN/516 and including the names of two academic referees should reach the Personnel Officer by 15th October, 1990.

EQUAL OPPORTUNITIES EMPLOYER

(4691)A

Cancer Research Campaign

PATERSON INSTITUTE FOR CANCER RESEARCH, Christie Hospital & Holt Radium Institute, Manchester

GENE REGULATION IN LEUKAEMOGENESIS

Three newly created posts, supported by the Cancer Research Campaign, are vacant in an established group which has recently moved to the Paterson Institute from the Royal Free Hospital School of Medicine, London. The interests of the group centre on various aspects of the regulation of gene expression and molecular mechanisms of leukaemogenesis. The area of research undertaken by the persons appointed to these posts will be decided by mutual arrangement.

POSTDOCTORAL SCIENTIST

Applications are invited from postdoctoral scientists, preferably with expertise in the field of molecular biology. The successful candidate will be encouraged to work independently with a minimum of supervision. However, more junior candidates are also urged to apply since training and supervision can be given. The position is tenable for up to 5 years in the first instance with an initial salary within the range £13,495 p.a. plus a £1,249 p.a. non-superannuable supplement, to £16,755 p.a. plus a £1,410 p.a. non-superannuable supplement.

HIGHER SCIENTIFIC OFFICER

The person appointed will be expected to provide technical support for one or more projects in the research programme, in addition to assisting in the running of a busy molecular biology laboratory. The successful candidate should have a BSc or HNC in a biological subject, together with several years laboratory experience. Some expertise in molecular biology and cell culture techniques would be an advantage. The appointment will be in the salary range £10,678 - £14,909 p.a. (pay award pending). In addition a 6% non-superannuable supplement is payable to members of the NHS superannuation scheme.

RESEARCH STUDENTSHIP

Applications are invited from enthusiastic, recent graduates in a biological subject for a 3 year Studentship leading to a PhD degree of the University of Manchester. This position will be of interest to candidates wishing to acquire a thorough training in techniques of molecular biology. Applicants should have at least an upper second-class honours degree. The Studentship will be supported through a CRC stipend of £6,000 in the first year, £6,500 in the second year and £7,000 in the final year.

Applications, including a full CV and the names of two academic referees, should be directed to the Laboratory Administrator, Paterson Institute for Cancer Research, Christie Hospital & Holt Radium Institute, Wilmslow Road, Manchester M20 9BX (Tel. 061-445 8123, Ext. 407). For informal enquiries, please contact Dr John Norton (Ext. 402).
The closing date for applications is Friday 12th October 1990. Prospective applicants should note that smoking is strongly discouraged in this Institute.

Fighting cancer on all fronts.

(4687)A

the John Radcliffe
OXFORD

immunologist to oxford transplant centre

Salary up to £36,457.

Applications are sought for the above Post which is a Senior NHS Post in Immunology responsible for the Clinical Immunological Service in the Oxford Transplant Centre under the direction of the Director of the Centre, Professor P. J. Morris.

The person appointed would be responsible not only for the supervision of the Tissue Typing Unit (previously directed by Dr Alan Ting) but would play a key role in initiating clinical research and bringing more basic development in Immunology to Clinical Trial. A new Oxford Transplant Centre is to open at the end of 1990 at the Churchill Hospital and will include a laboratory suite for Immunology and in particular, Tissue Typing. The successful appointee will also be expected to play an active role in the more basic Transplantation Immunology Research Programme which will remain within the Nuffield Department of Surgery Laboratories at the John Radcliffe Hospital.

A salary of up to £36,457 per annum is available depending upon qualifications and experience. In addition, an excellent relocation package may also be available where appropriate.

Informal enquiries are welcomed, please contact Professor P. J. Morris, Nuffield Department of Surgery, John Radcliffe Hospital on Oxford (0865) 817568 or if after the 29th September, Oxford 221297.

For an Information Pack and Application Form please contact Mike Fleming, Director of Personnel, Level 3, John Radcliffe Hospital, Headington, Oxford. OX3 9DU. Telephone (0865) 817520 or if after the 29th September, Oxford (0865) 221383.

Closing date: 9 November 1990.

(4670)A

Oxfordshire
Health Authority
AN EQUAL OPPORTUNITY EMPLOYER

CSIRO DIVISION OF HORTICULTURE
POSTHARVEST RESEARCH LABORATORY, SYDNEY
PLANT PHYSIOLOGY/PLANT MOLECULAR BIOLOGY

RESEARCH SCIENTIST – PRINCIPAL RESEARCH SCIENTIST

A$33,445 – A$56,887

The CSIRO Division of Horticulture seeks a scientist with expeience in plant molecular genetics to participate in research on the biology of maturation and senescence in fruits and vegetables in its Sydney laboratory.

The Division: The Division conducts applied and strategic research on horticultural crops of economic importance to Australia. Activities extend from production to postharvest studies on a range of temperate subtropical and tropical fruits, vegetables and ornamentals.

The Job: The scientist will be involved in research identifying genes and gene products that regulate maturity or ripening in fruits with the aim of modifying ripening by genetic manipulation. The opportunity also exists for independent research initiatives in a related area.

The Person: Applicants should have a PhD degree, or an equivalent qualification, in Plant Science, Biochemistry or Genetics and postdoctoral experience in molecular biology. An interest in horticulture would be an advantage. A person with an exceptional record in research may be appointed at the most senior level and be expected to exercise a research leadership role.

Conditions: This is an indefinite appointment with Australian Government Superannuation benefits.

More Information: Prospective applicants are invited to telephone Dr Colin Brady (612) 805 8219 for further information and Mr Rick Wright (612) 887 8336 for a duty statement and selection criteria.

Applications: Applications close 19 October 1990 and should quote reference number N90/1/N. They should relate to the selection criteria and provide details of relevant personal particulars. Applicants should nominate at least two professional referees and address their application to:

The Chief (N90/1/N)
CSIRO, Division of Horticulture
GPO Box 350
ADELAIDE S.A. 5001
AUSTRALIA

(W7747)A

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AB1/GH40N

DEPARTMENT OF RESOURCE ENGINEERING

Associate Professor/ Senior Lecturer

The Department is one of the core departments of the School of Natural Resources and has teaching and research interests in the broad areas of water resources management, water and soil conservation engineering and agricultural engineering. The Department teaches courses for the B.Nat.Res., B.Urb.Reg.Plan., B.Rur.Sci., and M.Nat.Res. degrees and from 1991 offers a new degree in Resource Engineering.

Appointee will teach courses and supervise postgraduate studies in aspects of water resources managment and water and soil conservation and may assist with basic courses in engineering science and practice. Appointee will play a major part in planning and development of new courses and laboratory facilities for the B.E degree programme. An appointee at Associate Professor level will be expected to take a substantial role in department administration and to serve as Head of Department from time to time.

Applicants should hold a first degree in civil or agricultural engineering and have appropriate postgraduate qualifications. Teaching and research supervision experience in a tertiary institution is essential and professional experience in water resources management or water and soil conservation engineering is desirable.

Informal enquiries to Prof. J. Burton. (ISD 61 67 73 2668).

SALARY: A$57,493 per annum (Associate Professor).
A$43,984-$51,015 per annum (Senior Lecturer).

CLOSING DATE: 12th October 1990.

POSITION No: 596.

Applications, should be addressed to the Head, Academic Staff Services, Human Resource Management Centre, University of New England, Armidale 2351 AUSTRALIA (FAX (ISD 61 67) 73 2755) and include names and addresses of three referees and state position number. Referees should be given a copy of this advertisement and asked to write direct to the Human Resource Management Centre by the closing date and quote the position number.

UNE
The University of
NEW ENGLAND
Armidale

Armidale, NSW 2351. Australia
Facsimile (067) 73 2755

(W7750)A

Equal opportunity is University policy

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS

Department of Experimental Pathology

Division of Histopathology

RESEARCH ASSISTANT (GRADE IB)

to work on human atherosclerosis. Research will involve immunohistochemical and in-situ hybridisation studies on the cells of the lesions, particularly in relation to the expression of cytokines and adhesion molecules. Experience of hisotological, immunological or molecular biological techniques an advantage. Based on Guy's Campus, the post is available immediately. Registration for a PhD would be considered.

Salary in the range £13,166-£13,853 per annum inclusive. Informal enquiries to Dr R Poston on 071-955 4785.

Applications in the form of full CV (2 copies) and the names and addresses of two referees should be sent ot the Personnel Officer, UMDS, Lambeth Palace Road, London SE1 7EH, quoting ref no G/HISTO/602 by 5th October, 1990.

(4648)A

UNIVERSITY OF EDINBURGH

Department of Veterinary Pathology

IMMUNOLOGIST AND IMMUNOCHEMIST

Applications are invited for two three year appointments funded by the Argricultural and Food Research Council for the biochemical and functional characterisation of the bovine MHC class II molecules. This is an AFRC Linked Research programme between the Department of Veterinary Pathology, University of Edinburgh and the Department of Immunogenetics at the AFRC Institute of Animal Physiology and Genetics.

Postdoctoral research fellow, grade AR1A (salary £13,495 – £14,744) with experience of cellular immunology, including the production of monoclonal antibodies. Experience with general immunochemical techniques would be advantageous. The appointee will be concerned with the immunogenetics of bovine MHC class II and its role in responses of cattle to synthetic peptides derived from VP1 of foot-and-mouth disease virus. Applicants should possess a PhD and preferably have three years postdoctoral experience. (POST REFERENCE NO.5814).

Postgraduate research associate grade AR1B (salary £11,339 – £12,792), with a first or second class degree in a scientific subject and preferably some biochemistry/immunochemistry experience. The post will be concerned with the immunochemical characterisation of the bovine MHC class II moelcules using monoclonal antibodies (POST REFERENCE NO. 5814A).

The successful applicants will be joining vigorous and expanding teams of scientist studying the molecular and cellular aspects of the immune response in cattle and sheep.

Informal enquiries may be made to Dr John Hopkins, Dept. of Veterinary Pathology, University of Edinburgh, Summerhall, Edinburgh. EH9 1QH. Telephone 031-667 1011, extension 5202. Further particulars of both posts are available from the Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh, with whom applications by letter, including cv and the names and addresses of two referees should be lodged not later than 11th October, 1990.

Please quote the relevant post reference number.

(4637)A

UNIVERSITY OF EDINBURGH **Department of Pharmacology**

POSTDOCTORAL FELLOW

Applications are invited from candidates with some experience of intracellular recording techniques and an interest in either a single electrode voltage clamp or patch clamp recording from thin slices or acutely dissociated neurones. Interest of the group, consisting of five post-doctoral fellows is concentrated on the 5-HT_{1A} receptors of the dorsal raphe nucleus and the role of auto receptors in transmitter release. The laboratory is generously equipped with the appropriate amplifiers, interfaces and computers. The group includes synthetic chemists and is involved in the preparation of SPET ligands for use in man.

The post is available for 3 years in the first instance and the basic starting salary could be £14,038 for a researcher aged 28 plus an enhancement premium of two incremental points making a total remuneration of £15,444 p.a.

Applicants should send a full curriculum vitae with the names of two referees to **Professor J.S. Kelly, Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ,** from whom further details can be obtained (Tel: 031-667 1011 x2358 or Fax: 031-667 9381). *PLEASE QUOTE REFERENCE NO. 5821.*

(4655)A

UNIVERSITY OF LONDON

Institute of Psychiatry, Departments of Genetics and Neuroscience

POSTDOCTORAL RESEARCH WORKER

A three position is available to work on the development of repeat sequence DNA polymorphisms at candidate gene loci for use in association and linkage analysis of the major psychoses. The successful candidate would join an expanding team of scientists and clinicians involved in molecular biological research, and would be responsible for the isolation and testing of new polymorphic microsatellite repeat sequences. Some experience in human genetics would be advantageous but not essential.

Applications in the form of a detailed cv including the names and addresses of two referees, quoting Reference 90/R34, should be sent to the Personnel Office, Institute of Psychiatry, DeCrespigny Park, London SE5 8AF, not later than 12th October, 1990. Informal enquiries and further details can be obtained from Dr M Gill or Dr J Powell on 071-703 5411 ext 3343/ 3536.

(4677)A

CHIEF POSITRON EMISSION TOMOGRAPHY

Intramural Research Program
National Institute of Mental Health

The NIMH is accepting applications for a senior neuroscientist with experience and interest in applying PET technology to the problem of brain function and structure and specifically to the localization of brain function in relation to behavior, cognition and mental disorders.

Applicants must hold a doctoral degree (M.D. or Ph.D.) or equivalent in one of the clinical or basic neuroscience disciplines, e.g., neuropsychiatry, psychiatry, neurology, neuropsychology, neuro-physiology, developmental neuro-biology, etc.

The NIMH PET laboratory is located in NIMH Intramural facilities at the Clinical Center of the National Institutes of Health (NIH) in Bethesda, Maryland, and the research is carried out with the technical support of the NIH Nuclear Medicine Department. Two dedicated head scanners are shared among the three main user Institutes and substantial scanning time and resources are available. (Nuclear Medicine operates the cyclotrons, PET scanners, and supplies radiochemistry services for imaging research to all user Institutes.) Opportunities for collaboration in human studies abound, and facilities for basic animal research are also available.

Duties will include scientific leadership, administration and coordination of the NIMH PET program as well as the conduct of internationally-recognized research. Salary will be commensurate with that of other NIMH investigators with equivalent qualifications and responsibilities, ranging from $50,342 to $78,200 plus full Federal benefits and, for M.D.'s only, relevant bonuses or special pay. U.S. citizenship required.

Send C.V., bibliography, a statement of future research directions, and the names of three references to:

Steven M. Paul, M.D.
Director
Intramural Research Program
National Institute of
Mental Health
Building 10, Room 4N-224
9000 Rockville Pike
Bethesda, MD 20892

The NIMH is an Equal Opportunity Employer.

(NW5791)A

Postdoctoral Positions

Yale University School of Medicine

The Radiobiology group has postdoctoral research opportunities in the areas of DNA repair, recombination and mutagenesis, and oncogene action. Faculty and their research interests are listed below:

1. Douglas E. Brash — Mechanisms of sunlight-induced human skin cancer: excision repair at the DNA sequence level *in vivo*. Oncogenes activated in human skin cancer by sunlight.
2. Franklin Hutchinson — Analyses of experimental data in molecular radiation biology, including data on hotspots in mutagenesis. Determinations of the structures surrounding various mutagenic products in DNA, using the facilities of the Yale Center for Structural Biology.
3. Barry Kacinski — The roles of lympho-hematopoietic factors and receptors in the invasive phenotypes of macrophages, certain carcinomas and implanting trophoblast, and in cellular resistance to DNA damaging agents.
4. R. Michael Liskay — Molecular mechanisms of genetic recombination and repair in mammalian cells. Isolation of mammalian genes involved in recombination and repair.
5. K. Brooks Low — Genetic and molecular analysis of homologous recombination in bacterial systems, including damage-stimulated recombination. The role of mismatch repair in recombination.
6. Charles M. Radding — The enzymology of homologous recombination with special interest in the fundamental mechanisms of homologous pairing and strand exchange, but also in practical uses of recombination enzymes for molecular biology and biotechnology.
7. W. Dean Rupp — Molecular mechanisms of DNA repair. The systems range from whole cells to highly purified enzymes, and use specifically constructed DNA substrates.
8. William C. Summers — Mammalian cell responses to DNA damage. Mutagenesis and repair of DNA *in vivo*.

Inquiries and applications, including curriculum vitae, brief description of research interests and names of three references should be directed to:

Ms. Celeste Soli
Radiobiology Labs
Yale University School of Medicine
P.O. Box 3333
New Haven, CT 06510

Yale University is an Affirmative Action/Equal Opportunity Employer

(NW5797)A

Yale UNIVERSITY

Developmental Geneticist

The Department of Biology at Yale University seeks to appoint a Developmental Geneticist at the level of Assistant Professor (Ladder Faculty). Applications are invited from individuals using classical and molecular genetic approaches to study development. The successful candidate is expected to carry on a vigorous, independent research program, supervise the research of graduate students and postdoctoral fellows, and contribute to undergraduate and graduate teaching.

Please send curriculum vitae, description of research interests and the names and addresses of at least three referees by January 1, 1991 to: **Developmental Geneticist Search Committee, c/o Chairman, Department of Biology, Yale University, P.O. Box 6666, New Haven, CT 06511-8112.**

Yale University is an Affirmative Action/ Equal Opportunity Employer.

Women and members of minority groups are especially urged to apply.

(NW5790)A

Postdoctoral Research Position in Tropical Palynology

Smithsonian Tropical Research Institute

The Smithsonian Tropical Research Institute invites applicants for a 3 year postdoctoral position in paleoecological palynology to start in July 1991. The Institute is a bureau of the Smithsonian Institution and has headquarters in the Republic of Panama. The Center for Paleoecology at STRI is documenting the environmental and ecological history of the region, including anthropogenic influences on ecosystems. Investigators participating in the work of the Center include: Paul Colinvaux, Richard Cooke, Jeremy Jackson, and Dolores Piperno. Annual stipend starting $25,000 plus travel, benefits, and research support. Screening will begin on 1st January, 1991 and continue until the position is filled. Applicants should include statement of research interest, curriculum vitae, reprints, and names, addresses, and telephone numbers of 3 references. Send to: **Palynology Search Committee, Smithsonian Tropical Research Institute, APO Miami 34002-0011, USA.**

The Smithsonian Institution is an Equal Opportunity/Affirmative Action Employer, and appointment can be made without regard to nationality.

(NW5798)A

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

Initial salary within the range £11,399-£13,495 p.a.

Tenable for three years from 1 December, 1990.

To work on a project entitled "Identification of Proteins Intimately Associated with Proteins in the Act of Translocation" which seeks to answer fundamental questions about the mechanisms of protein translocation and membrane protein integration.

The project will develop expertise in biochemical, microbiological, genetic and molecular biology techniques and applicants with expertise in one or more of these areas are encouraged to apply.

Informal enquiries to Dr. J. M. Pratt on 051-794 4322.

Telephone numbers of referees should also be included.

Quote ref: RV/817/N

DEPARTMENT OF GENETICS AND MICROBIOLOGY

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

Initial salary in the range £11,399-£12,792 p.a.

Funded by the Agricultural and Food Research Council as a three-year project on the characterisation and function of bacterial extracellular peroxidases.

The successful candidate will join an active group of research scientists in this well-equipped department. Applicants should have or be about to obtain a Ph.D. in microbiology, biochemistry, molecular biology or a related subject. Some experience in enzymology and/or protein biochemistry would be advantageous.

The research programme will be directed by Dr. A. J. McCarthy and Dr. P. G. G. Miller, to whom informal enquiries maybe made on 051-794 4413 or 051-794 3586.

Quote ref: RV/818/N

Closing date for the above two posts: 4 October, 1990.

Applications, by c.v. with the names of three referees, should be received by the Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained.

(4680)A

University of LIVERPOOL

AN EQUAL OPPORTUNITY EMPLOYER

UNIVERSITY OF BRISTOL

Department of Geology

RESEARCH ASSISTANT
RESEARCH TECHNICIAN

Applications are invited for a Postdoctoral Research Assistant and a Research Technician to work with Drs R J Parkes, J C Fry (UCW at Cardiff) and R A Herbert (Dundee University) on a two year NERC-funded project on the isolation and characterization of bacteria within deep sediment layers collected on recent Ocean Drilling Programme cruises. This research is part of a major new biogeochemistry initiative in the University.

Candidates for the PDRA post should have experience in the isolation and cultivation of bacteria, particularly anaerobic bacteria, and in the analysis of bacterial metabolic products and cellular components. A knowledge of bacterial taxonomy or sediment microbiology would be an advantage. The Research Technician should be experienced in cultivation of microorganisms and basic analysis of microbial growth and metabolism.

Salary PDRA on RA1A scale, £11,399 – £18,165 per annum, Research Technician Grade B £7,970 – £8,453 per annum.

Informal enquiries may be made to **Dr R J Parkes, Department of Geology, Bristol (0272) 303154.**

For further details telephone Bristol 303136 (ansaphone after 5.00 pm) or write to the Personnel Office, Senate House, Bristol, BS8 1TH. *Applications should be sent to that address as soon as possible. Please quote reference 425.*

An Equal Opportunities Employer.

(4634)A

UNIVERSITY OF MICHIGAN

Department of Geological Sciences

ASSISTANT PROFESSOR
(Tenure-track) in Igneous Petrology

The Department of Geological Sciences invites applications for a tenure-track position in igneous petrology at the Assistant Professor level starting September 1991, pending University approval of the post. A Ph.D. degree is required. The position will involve development of a research program in igneous petrology, as well as teaching at the undergraduate and graduate level. Research specialties may include any aspect of igneous petrology: field, analytical, experimental or theoretical, with some preference to applicants with a broad range of interests. The Department is equipped with extensive modern analytical facilities, including XRF, XRD, SEM, TEM, electron microprobe, ICP, and radiogenic and stable isotope geochemistry laboratories.

Applicants should send a copy of their curriculum vitae, a brief description of their research and teaching interests, and the names, addresses and telephone numbers of five academic references from whom the Department may request letters of recommendation.

Documents should be sent to Professor Eric Essene, Search Committee Chairman, Department of Geological Sciences, University of Michigan, 1006 C.C. Little Building, Ann Arbor, MI 48109-1063, telephone (313) 764-8243, FAX (313) 763-4690, Bitnet Essene@UM.CC.UMICH.EDU to arrive by 1st January, 1991. The University of Michigan is an Equal Opportunity/ Affirmative Action Employer.

(NW5785)A

THE UNIVERSITY OF SHEFFIELD

Department of Physics

EXPERIMENTAL OFFICER/SENIOR EXPERIMENTAL OFFICE

OPTICAL PROPERTIES OF SEMICONDUCTOR LOW DIMENSIONAL STRUCTURES AND DEVICES

Applications are invited for the above newly created permanent post tenable from January 1991. The appointee will play a major role in a new experimental programme on the optical properties of semiconductor low dimensional structures and devices which is being set up at Sheffield under Professor-elect M S Skolnick and Dr D J Mowbray. The activities will involve a wide range of optical and electrical techniques (photoluminescence, photoconductivity, magneto-optical experiments, Raman scattering etc) on state-of-the-art low dimensional structures prepared principally in the Department of Electronic & Electrical Engineering at Sheffield.

Salary according to age and experience within Other Related Grade 1 (£11,399-£15,444 pa) or Grade 2 (£14,038-£18,165 pa).

Informal enquiries to Dr M S Skolnick on 0684 894080.

Applications, including a full CV and the names/addresses of three referees (six copies of all documents) should be sent to the Director of Personnel Services, The University, Sheffield S10 2TN by 12th October, 1990. Please quote ref: R.1050/G.

AN EQUAL OPPORTUNITY EMPLOYER

(4646)A

JUNIOR POSITION in BIOPHYSICAL CHEMISTRY

Biocenter of the University of Basel, Switzerland

One of our two vacant positions has been filled with a scientist working on membrane fusion. For the second opening applications are invited from younger persons who can carry out independent research of high quality. Projects dealing with the physical chemistry of either biomembranes or proteins would be of particular interest. The conditions of appointment will depend on qualifications and performance. The department offers access to a wide variety of modern instrumentation as well as opportunities to interact with many groups doing biological research at the molecular and cellular level.

Applicants should send a letter including curriculum vitae, the names and addresses (with phone and FAX numbers) of two persons willing to act as referees, list of publications and brief statements describing past and present research activities as well as future plans to **Professor J Engel, Biocenter, Department of Biophysical Chemistry, Klingelbergstrasse 70, CH-4056 Basel, Switzerland.**

Deadline: **31st December, 1990.**

(W7752)A

Senior Research Biologist

Glaxo Group Research has a considerable, growing commitment and research effort aimed at the discovery and development of effective new medicines for the cure of the common cancers of Man. Our innovative approach has made us leaders in the prevention of emesis associated with cancer chemotherapy and one of the world's leading pharmaceutical research organisations.

Our Cell and Molecular Science Division is currently establishing two new research teams with the specific remit to explore new areas in the cell and molecular science of cancer. This is a major new initiative which involves investment and collaboration on an international basis. These groups will require the appointment of group leaders and the positions will provide an ideal career launch opportunity for the established scientist.

One of the teams will have a bias towards Cell Biology and will be concerned with the testing of novel chemical approaches and "cutting edge" tools, including antisense and triplex forming oligonucleotides, which will permit modification of genes and their activities. The other team will help us gain an understanding of the genetic and molecular changes which lead to the development of the common tumours in Man and thus provide novel molecular targets which will give therapeutic discrimination between normal and neoplastic tissue. Initially the work will involve development of *in vitro* systems which can be translated to *in vivo* for the study of mutations in important genes and loss of suppressor control.

The successful applicants will have had considerable involvement in the application of Molecular Biology to Biochemistry or Cell Biology, proven by experience and publication record. They will be innovative practical scientists capable of defining a research programme of work and skilled in communicating their ideas. There will be considerable liaison between the research teams and other teams in the Company and Academia. The work of the two teams is complementary and so the precise make up of the teams and team leaders will be balanced to provide a strong research group. The team leaders will be responsible for the attainment of research goals and the supervision and motivation of their teams in a stimulating and challenging research environment.

The rewards packages are outstanding and include an impressive range of benefits and, where relevant, relocation assistance.

Please send or fax full cv or telephone for an application form to Cathy Woodbine,
Personnel Department, Glaxo Group Research,
Greenford Road, Greenford, Middlesex UB6 0HE.
Tel: 081-422 3434 ext. 2573. Fax 081-422 4205.

Closing date: 11th October 1990. (4688)A

Glaxo Group Research

THE UNIVERSITY OF SHEFFIELD
Department of Animal & Plant Sciences

TWO POSTS FOR ORNITHOLOGISTS

RESEARCH ASSISTANT AND FIELD ASSISTANT

Applications are invited from persons with an interest in Ornithology for two posts:

Research Assistant to work with Dr T R Birkhead on a 3-year laboratory-based project on sperm competition in birds. Applicants should have a good degree in Zoology and/or related discipline and some experience of working with birds would be useful. Starting salary £11,399 pa.

Field Assistant to work on a population study of guillemots on Skomer Island with Dr T R Birkhead between April-July 1991. Some field experience with seabirds would be useful.

Further information from **Ms A Sellars, Department of Animal & Plant Sciences, The University, Sheffield S10 2UH,** to whom applications, including a CV and the names/addresses of 2 referees should be sent by 12th October, 1990. Ref MAP 321/G.

AN EQUAL OPPORTUNITY EMPLOYER

(4671)A

Gesellschaft für Schwerionenforschung, Darmstadt (GSI)
und
Ruprecht-Karls-Universität Heidelberg

Bei der GSI ist zum frühestmöglichen Zeitpunkt die Position eines/einer

LEITENDEN WISSENSCHAFTLERS/IN FÜR DEN FORSCHUNGSBEREICH ATOMPHYSIK

ZU BESETZEN.

Der/die Leitende Wissenschaftler/in soll mit Zustimmung des Landes Baden-Württemberg zugleich Professor/in (Bes. Gr. C 4) für Experimentalphysik (Physik hochionisierter Atome) an der Universität Heidelberg werden und das Arbeitsgebiet Experimentelle Atomphysik mit schweren Ionen in Forschung und Lehre vertreten. Er/sie soll einen Forschungsbereich bei der GSI leiten, der insbesondere Experimente an dem Schwerionensynchrotron (SIS) und dem Speicherring (ESR) zur Untersuchung der Wechselwirkung von hochgeladenen Ionen mit Teilchen und Photonen durchführt. Seine/ihre Lehrtätigkeit soll im Rahmen der Fakultät für Physik und Astronomie der Universität Heidelberg erfolgen. Der/die Stelleninhaber/in wird nach Ernennung zum Professor an der Universität Heidelberg zur GSI beurlaubt werden.

Bewerbungen werden bis 15.11.1990 erbeten an:

Herrn Prof. Dr Paul Kienle
Wissenschaftlicher Geschäftsführer der
Gesellschaft für Schwerionenforschung mbH
Planckstr. 1 — Postfach 11 05 52
6100 Darmstadt 11

(W7755)A

Leicester University

DEPARTMENT OF BIOCHEMISTRY & BIOLOGICAL NMR CENTRE

POST-DOCTORAL RESEARCH ASSOCIATES

NMR SPECTROSCOPY OF PROTEINS: ANALYSIS OF THE EFFECTS OF SITE-DIRECTED MUTAGENESIS

As part of a programme of research into the structure, mechanism and inhibition of dihydrofolate reductase, two positions are available for nmr studies of the enzyme in the laboratory of Prof G C K Roberts. This enzyme is the target for a number of useful drugs and, in collaboration with Drs J Feeney (Mill Hill) and R Hubbard (York), we are applying site-directed mutagenesis, nmr and molecular modelling methods to study its structure and the molecular basis of its specificity in binding substrates and inhibitors (see, eg, Protein Eng., 2, 627, 1989; Biochemistry, *28*, 1353 & 5743, 1989). The project will involve the application of modern 2D and 3D nmr methods, including the use of isotope labelling, to the study of site-directed mutants of the enzyme, comparing their structure and interactions with those of the wild-type. It will also provide the opportunity to gain some experience of molecular modelling and mutagenesis. Further information available from Prof Roberts (0533-522978).

The positions, funded by the Wellcome Trust and SERC, are for three years from 1st October, on salary range RA IA (£11,399-£18,165) according to age and experience; the starting date could be delayed for an exceptional applicant. Applicants should have experience in modern nmr methods, though not necessarily in their applications to proteins.

Applications, including full CV and the names of two referees, should be sent to **Professor G C K Roberts, Biological NMR Centre, University of Leicester, PO Box 138, University Road, Leicester LE1 9HN.**

Closing date for receipt of applications: 20th October, 1990.

(4653)A

INSTITUTE OF ZOOLOGY — ZOOLOGICAL SOCIETY OF LONDON

HEAD OF CONSERVATION BIOLOGY

A senior population biologist is required to head the Institute's Conservation Biology Research Group.

The Group, which currently comprises 7 members of scientific and technical staff in molecular and population genetics, carries out basic studies on the genetic structure of small populations and provides practical advice in the Society's field conservation projects. Current post holder is Dr. G M Mace. The ideal candidate will be an experienced population biologist with expertise in genetics, demography or field ecology, combining sound academic ability and experience with a broad knowledge of global species conservation priorities. He or she will be capable of administering a young and expanding research group partially dependent on external funds.

The Insitutute of Zoology is a grant-aided Institute of the University of London and there are opportunities for undergraduate and postgraduate teaching. In addition the post holder will be expected to tutor students from developing countries attending the Institute for postgraduate wildlife veterinary training. Salary on the University Lecturer/Senior Lecturer scale in the range £19,614 – £26,472 plus London Weighting according to age and experience. The contract will initially be for 5 years and renewable.

Further details from Professor A P F Flint, Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY (tel: 071-722 3333; fax 071-483 4436), to whom written applications, with detailed CV, publications list and names of three academic referees, should be sent, not later than 18th October, 1990.

(4639)A

University of Cambridge

DEPARTMENT OF ZOOLOGY

University Lectureship or University Assistant Lectureship

Applications are invited for a Lectureship or Assistant Lectureship in the Department of Zoology. Applicants should have a wide-ranging interest in animal physiology and an active research programme in this area. The Department has a strong interest in many aspects of biological science, including invertebrate and vertebrate physiology and ecology.

The appointment will be for three years, with the possibility, for a University Lecturer, of reappointment to the retiring age or, for a University Assistant Lecturer, of reappointment for two years. The statutory limit of tenure of a University Assistant Lectureship is five years, but all holders of the office of University Assistant Lecturer are considered for possible appointment to the office of University Lecturer during their course of tenure. The scale of stipends for persons not ordinarily resident in College are, for a University Lecturer, £15,444 pa rising by 11 annual increments to £23,819. There is no grade of Senior Lecturer. For a University Assistant Lecturer, £12,086 a year rising by seven annual increments to £16,755.

Please ask for further information on the post and the Department from John Andrews, Secretary to the Appointments Committee of the Faculty of Biology 'A', Department of Zoology, Downing Street, Cambridge CB2 3EJ. Tel: 0223 336621, Fax: 0223 336676 (International +44 223 336621 and 336676). Applications (12 copies) should include a *curriculum vitae*, list of publications, statement of intended research and the names of not more than three referees. The closing date is 5th November.

The University of Cambridge follows an equal opportunities policy.

(4645)A

BEHAVIORAL NEUROSCIENCE SPECIALIST

PhD in Behavioural Neurosciences with emphasis in Human Behavioral Neurosciences (12 credit hours). To be a member of the treatment team responsible for neuropsychological evaluation, neurobehavioral intervention, designing and implementing research to evaluate effectiveness of treatment programs for aphasic disorders and psychomotor impairment. Must be knowledgeable in Aphasia, Neuroassessment and Psychomotor Behavior (1 year hands-on experience, dissertation or 3 credit hours course work in each area). $43,000/yr, 40 hours per week.

Apply at the Texas Employment Commission, Galveston, Texas, or send resume to the Texas Employment Commission, TEC Building, Austin, Texas 78778, JO #5757206.

Ad paid by An Equal Employment Opportunity Employer. (NW5794)A

ASSISTANT PROFESSOR OF ANATOMY

Memorial University of Newfoundland invites applications for a tenure-track position of Assistant Professor of Anatomy in the Faculty of Medicine. The successful applicant would teach gross anatomy to medical students and would be expected to have, or to develop, a strong research program compatible with existing areas of strength (Neuroscience, Immunology, Molecular Biology). An ability to teach embryology, histology, or neuroanatomy would be an advantage. Excellent facilities for research are available, including electron microscopy, histology, computing services, and animal care, together with neuropharmacology, neurophysiology and clinical sciences. Funds will be made available to set up a research program. Assistance with relocation is available.

Memorial University of Newfoundland is an equal opportunity employer. In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

Applications, including a curriculum vitae, a statement of research interests, and the names of three references should be sent by 30th October, 1990 to: **Dr T M Scott, Professor of Anatomy, Faculty of Medicine, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3V6.**

(NW5799)A

RESEARCH ASSOCIATE
Department of Physiology
Faculty of Medicine

Effective Date:
1st January, 1991

Salary: $24,000 – $36,000 per annum

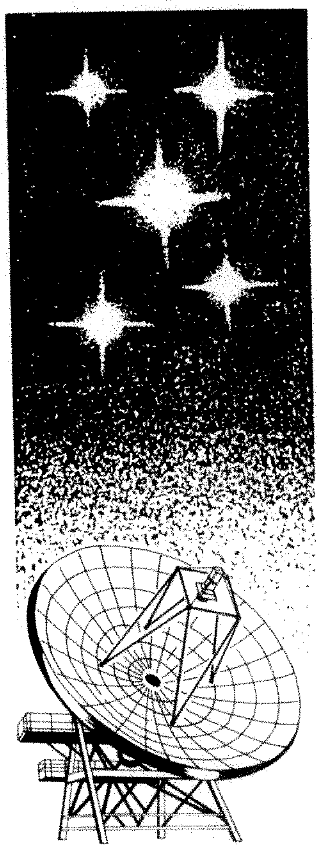
One position will be available January 1, 1991, for an individual to actively participate in research and to supervise the scientific program of a team consisting of over 40 members. A Ph.D. in Biomedical Sciences is required, and supervisory/administrative experience is desirable. Four major areas of investigation include: comparative vertebrate physiology, cellular calcium physiology, hypertension and herbal medicine. Expertise in all areas is preferred.

Please send curriculum vitae and two letters of reference before 31st October, 1990, to: Dr. Peter K.T. Pang, Professor and Chairman, Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7.

The University of Alberta is committed to the principle of equity in employment. The University encourages applications from aboriginal persons, disabled persons, members of visible minorities and women. In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

(NW5787)A

HIGHER SCIENTIFIC OFFICER
MILLIMETRE-WAVE ASTRONOMER/SCIENTIST



The Royal Observatory, Edinburgh operates a submillimetre telescope, the James Clerk Maxwell Telescope (JCMT), in Hawaii, on behalf of the astronomical communities in the UK, Canada, the Netherlands and Hawaii. This operation is supported by a group in Edinburgh where there is a vacancy for an astronomer or instrument scientist.

The successful candidate will be required to take some responsibility for the management of the JCMT Edinburgh operations, provide advice to users on aspects of the telescope's instrumentation and participate in the development of JCMTs instrumentation programme. The appointee may expect to be transferred to the operating team in Hawaii at some stage for a tour of duty.

Applicants must possess a good degree in physics, astronomy or a related subject and should have a broad knowledge of millimetre/submillimetre astronomy, including experience of receivers and making observations. The passing of a high altitude medical is a pre-requisite for this post.

Appointment will be to the grade of Higher Scientific Officer and may be permanent or, if more appropriate, fixed-term for a minimum of 2 years. Salary will be in the range £11,586 p.a. to £16,176 p.a. dependent on qualifications and experience. Further performance related increments up to £19,681 p.a. may be payable. Some assistance with specific relocation expenses may also be available.

The Observatory operates a "no smoking policy", is an equal opportunity employer and offers good conditions of service including a non-contributory pension scheme.

For further information and an application form contact Susan Punton (quoting ref 90/8) at the Royal Observatory, Blackford Hill, Edinburgh EH9 3HJ. Tel: 031-668 8268. Fax: 031-668 8264.

CLOSING DATE for the receipt of applications or C.V.'s: FRIDAY 2 NOVEMBER 1990.

(4679)A

serc

ROYAL OBSERVATORY, EDINBURGH

DALHOUSIE UNIVERSITY
Faculty of Medicine
Nova Scotia Medical Research Fund Basic Science Scholarship

The Faculty of Medicine announces a competition for one tenure-track appointment at the level of **Assistant Professor** to be made initially for three years through a Nova Scotia Medical Research Fund (NSMRF) Scholarship. This award will be made in one of the basic science (pre-clinical) departments including Community Health and Epidemiology however, a cross appointment in a clinical department will also be expected. The successful applicant must have a PhD, MD, DVM, or equivalent with recent postdoctoral experience, and is expected to develop an independent interdisciplinary research program with external funding, ideally in one of the major research areas (Cancer Cell Biology, Cardiovascular, Immunology & Infectious Diseases, Neuroscience) within the Faculty.

Applications consisting of a curriculum vitae, a summary of research interests and three letters of reference should be sent to: **Dr D Howard Dickson, Associate Dean Research, Faculty of Medicine, 15th Floor Tupper Medical Building, Dalhousie University, Halifax, Nova Scotia B3H 4H7.**

Closing date for receipt of applications is 4th January, 1991. This appointment will be made on or after 1st April, 1991. This position will be filled in accordance with the Dalhousie University Affirmative Action Policy. In compliance with Canadian Immigration Regulations, this advertisement is directed at this time only to Canadian citizens and landed immigrants.

(NW5806)H

PHARMACOLOGISTS
UNIVERSITY OF TORONTO

The Department of Pharmacology, Faculty of Medicine, University of Toronto invites applications for 3 tenure-track Assistant Professorships from individuals well-qualified to establish an independent first-class research program in one of the following areas.

#1. molecular biology of receptors/signal transduction/2nd messengers
#2. cellular/molecular toxicology
#3. molecular biology/genetics of drug metabolism

Highly qualified individuals working in other areas of pharmacology also are invited to apply. Successful applicants will have promise in teaching as well as in research and will be expected to teach some portion of pharmacology to students in undergraduate, graduate, or professional (medical/dental) courses and to train graduate students. Qualifications should include a PhD or MD degree with postdoctoral research experience. The starting date is July 1991 with some flexibility if necessary.

Applications should include a complete curriculum vitae, a letter stating current and intended research interests and the names of at least three referees. Please indicate which of the 3 specific positions you are seeking. Apply before 15th December, 1990 to: **Dr Allan B Okey, Professor and Chair, Department of Pharmacology, Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada M5S 1A8.**

The University of Toronto encourages both men and women to apply. In accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents of Canada.

(NW5801)A

The University of Sydney

AUSTRALIA

LECTURER

(Fixed Term)

Department of Veterinary Physiology

Reference No. 34/11

Applications are invited from graduates in veterinary science, agricultural science, rural science or science, with experience in the teaching of general physiology. Current research interests of the department are reproductive and digestive physiology and there are also opportunities for collaborative work with other departments. The appointee will be expected to teach undergraduates in the Faculties of Veterinary Science and Agriculture and also take part in postgraduate teaching. The position is a 5 year fixed-term due to the absence of a member of staff on secondment, and is available until December 1994.

Further information from Associate Professor Grant Stone (02) 692 2464, fax (02) 692 3957.

Salary: $33,163-$43,096 per annum.

Method of Application: Applications quoting Reference. No. and including curriculum vitae, list of publications and the names, addresses and fax nos. of three referees, (only three referees will be contacted in the first instance and if more referees are nominated they should be ranked in order of preference) to the Registrar, Staff Office, University of Sydney, NSW 2006 by 12 October, 1990.

(W7741)A

The University reserves the right not to proceed with any appointment for financial or other reasons.

Equal Employment Opportunity and No Smoking in the workplace are University policy.

60495

（株）アイ・シー・オー

薬事申請関連総合ドキュメンテーション

翻訳者（在宅）及びアドバイザー（在宅）募集

▶職　務：基礎（薬理・毒性・ADME等）及び臨床の翻訳、並びに薬事申請関連ドキュメンテーションのコンサルタント業務

▶資　格：医薬・農薬・動薬・化粧品・食品・医学に関する知識と語学力を有する方
年齢・性別不問

▶待　遇：出来高制

▶応　募：履歴書郵送又は持参　＊応募秘密厳守

▶連絡先：〒152 東京都目黒区鷹番3-6-9-405
〈担当：今〉TEL(03)712-2377
FAX(03)712-2414

大阪支店　〒541 大阪市中央区南久宝寺町4-5-11-606
〈担当：馬渕〉TEL(06)243-5980
FAX(06)243-5981

(W7451)A

Universität Heidelberg

POST-DOCTORAL POSITIONS

CONTROL OF TRANSCRIPTION AND mRNA PROCESSING MECHANISM OF ORGANELLE ASSEMBLY

Positions available at the Zentrum für Molekulare Biologie, University of Heidelberg, to work on: 1) the control of transcription and RNA processing in trypanosomes, including analysis of promotor function *in vitro* and by DNA transfection and 2) the mechanism of protein targeting to the glycosome, an unusual trypanosomal peroxisome-like microbody. Experience in molecular biology or cell biology required.

Please send cv, publications list (refereed publications in internationally recognized journals only; no abstracts please), description of research experience and names, addresses and telephone and/or Fax numbers of three referees to: Dr C Clayton, ZMBH, Im Neuenheimer Feld 282, D-6900 Heidelberg 1, Federal Republic of Germany (Tel: (06221) 566876; Fax (06221) 566809). (W7745)A

MEDICAL RESEARCH COUNCIL　HUMAN GENETICS UNIT
WESTERN GENERAL HOSPITAL, CREWE ROAD,
EDINBURGH EH4 2XU Tel: 031 332 2471

POST DOCTORAL SCIENTISTS

Three 3 year postdoctoral positions are available in the Structural Studies Section for work on the following projects.

1) Proteins involved in meiosis:

The project is designed to define and characterise proteins which are involved in mammalian meiosis using a PCR based method for isolation of cDNAs and immuno-histochemical methods of characterisation.

Once charaterised at the cytological level the genes for these proteins will be disrupted in mouse ES cells and the effects of the disruptions analysed using transgenic mice. (Ref: 978).

2) Centromere structure and function in S.pombe:

Two posts are available to work with Dr Robin Allshire on projects which involve the isolation and characterisation of genes/proteins required for centromere structure and function in S.pombe. Homologs of genes isolated will be actively sought and investigated in mammalian cells. These studies will involve both genetic and molecular biological analyses and the construction of new artificial chromosome cloning systems. (Ref: 979).

Applicants should have experience in molecular biology; yeast and or mouse genetics, protein biochemistry and immunocytochemistry experience would be an advantage. Further details available from Dr Howard Cooke at the above address.

Two 3 year post-doctoral positions are available, to work on the molecular genetics of X-linked retinitis pigmentosa. The appointed scientists will work within a larger group whose aims are the identification and characterisation of renal-expressed sequences in yeast artificial chromosomes. Applicants should hold or be about to obtain a PhD degree in molecular biology, genetics or a related discipline. The posts are funded by the National retinitis Pigmentosa and George Gund Foundations. (Ref: 980). Applications along with the names and addresses to two referees, to Ms M Marron, Unit Administrator. *The MRC is an Equal Opportunities employer.* (4662)A

THE UNIVERSITY OF BIRMINGHAM

Department of Physiology

RESEARCH FELLOW

To work in muscle biochemistry research group investigating the molecular basis of normal and abnormal muscle growth and function. Particular emphasis on role of polypeptide growth factors and contractile proteins. Wide range of techniques used in well equipped, newly established laboratories. Experience in molecular biology, protein biochemistry or immunochemistry advantageous. Post funded by the British Heart Foundation and available until November, 1991.

Salary on a scale £11,399 — £16,026.

Application forms, two copies (returnable by 10th October, 1990) and further particulars from the Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT. Tel. 021 414 6483 (24 hours). Quote ref: M3572.

The University is an equal opportunities employer. (4663)A

UNIVERSITY OF NOTTINGHAM

Department of Zoology

POSTGRADUATE RESEARCH ASSSISTANT

Applications are invited for a three year postgraduate research assistanship funded by the Science and Engineering Research Council. The project is concerned with the cause and effect relationship between individual differences in social behaviour and susceptibility to disease and will focus on the effects of social relationships in house mice on endocrine function, immune competence and response to infection with parasites. Candidates should possess a 1st Class or 2:1 Honours Degree in Zoology or a related subject and preferably have an interest in both behavioural and physiological aspects of Parasitology. The successful candidate will be working in the Animal Behaviour and Experimental Parasitology Research Groups in the Department. The salary will be in the 1B range £11,399-£13,495).

The closing date for applications is 1st November, and the post commences on 1st January, 1991.

Applications, together with a detailed curriculum vitae and the names of two referees, should be sent to Dr C J Barnard, Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD.

(4668)A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF PURE AND APPLIED BIOLOGY

RESEARCH ASSISTANT/ RESEARCH FELLOW MOLECULAR PARASITOLOGY (AFRC)

This post is available immediately for a fixed period of up to 15 months to work on the structure and immunogenicity of parasite surface proteins. The applicant will join a group working on cellular and molecular aspects of the important medical and veterinary parasite *Toxoplasma gondii*. The area of study is particularly suitable to the protein biochemist, immunologist or molecular biologist; both postdoctoral scientists and postgraduates are encouraged to apply.

Salary on the RA IB scale for Research Assistant (£11,399-£13,495) and on the RA IA scale (£11,399-£14,744) according to qualifications and relevant experience.

Informal enquiries may be made to Dr Judith Smith (tel (0532) 332892).

Application forms and further particulars may be obtained from and completed applications forwarded to the Registrar, The University, Leeds LS2 9JT (tel 0532 333969 — direct line) quoting reference no 56/37.

Closing date for applications: 11th October, 1990.

The University is an equal opportunities employer. (4673)A

Institute of Terrestrial Ecology

PLANT ECO-PHYSIOLOGIST BANGOR RESEARCH UNIT

SALARY UP TO £20,467

The Institute has a vacancy for a Plant Eco-Physiologist at its Bangor Research Unit, which is located within the University College of North Wales (UCNW).

This multidisciplinary Unit contains three small teams investigating aspects of:–

- montane ecology
- biogeochemistry as affected by land use change
- responses of semi-natural ecosystems to pollution.

A fourth section is being formed to study the effects of different land use systems in semi-arid zones on:–

- flora and fauna
- biogeochemical cycling
- hydrology.

This programme will be developed in collaboration with colleagues in the UCNW Centre for Arid Zone studies and the NERC Institute of Hydrology.

A Plant Eco-Physiologist with interests in stress tolerance mechanisms, and particularly the factors causing inter- and intra-specific variations in response and their effects on plant community structure, is required to play a leading role in the development of this collaborative programme. Candidates should have a proven record of research in this or a closely related field, preferably with overseas experience. Previous experience of managing contracted research would be valuable. Working knowledge of French will be an advantage.

Post-doctoral experience in eco-physiology and a driving licence are essential to this post.

The post will be for a period of three years, based at Bangor Research Unit.

Salary will be in the range £14,381pa to £20,467pa. Generous annual leave allowance and non-contributory pension scheme.

For further details and application form, please contact:– Suzanne Mitchell, Institute of Terrestrial Ecology, Monks Wood Experimental Station, Abbots Ripton, Huntingdon PE17 2LS. Telephone 04873 381, quoting Ref. No. ITE(S) 11990/91. The closing date for completed application forms is 11 October, 1990.

Natural Environment Research Council

The Institute of Terrestrial Ecology is a component body of the Natural Environment Research Council.

The Natural Environment Research Council is an equal opportunity employer.

(4685)A

GENETICISTS/ MOLECULAR GENETICISTS

Iowa State University is seeking candidates to fill several assistant/associate professor level faculty positions in molecular genetics. Successful candidates will be expected to establish a competitive research program and to participate in graduate and undergraduate education. Applications are encouraged from individuals interested in all areas of genetics, including animal, plant, microbial, population/evolutionary, or cytogenetics.

The successful candidates will have space in a new molecular biology building. Generous start up funds are available. The department contains active, collaborative research groups in genetics, neurobiology, developmental biology, cell biology, molecular biology, and physiology. ISU is an equal opportunity/affirmative action employer. Women and members of minority groups are encouraged to apply. Deadline for application is Dec. 17, 1990, or until the positions are filled. Candidates should send a current CV containing a description of research interests and arrange for three letters of recommendation to be sent to:

Dr. Jack R. Girton
Chair, Faculty Search Committee
Department of Zoology and Genetics
Iowa State University
Ames, Iowa 50011

(NW5810)A

INSECT BIOTECH
Canada

POSTDOCTORAL AND RESEARCH ASSOCIATE POSITIONS

Applications are invited for positions in BIOTECHNOLOGY applied to insects. The research program is directed toward the development of new biorational methods of insect pest control. Research areas include:

i) Molecular engineering of baculoviruses
ii) Molecular biology of neuropeptides
iii) Molecular genetics of pesticide resistance
iv) Cell and molecular biology of insects
v) Biosynthesis and action of juvenile hormone and analogs

Applicants should have a background in molecular biology and genetics and preferably some experience in insect physiology, biochemistry or toxicology. The salary scale for Postdoctoral Fellows will follow the guidelines set by the Natural Sciences and Engineering Research Council of Canada. In accordance with Canadian immigration requirements, priority for Research Associate positions will be given to Canadian citizens or permanent residents.

Insect Biotech Canada is a research consortium including twenty-five of the top Canadian scientists in this field in ten Universities and three Government laboratories across the country and includes industrial members. IBC is funded by the Government of Canada under the Networks of Centres of Excellence program.

Applicants should submit a *curriculum vitae*, names of three referees and a brief statement of research interests to: **Dr B J Hutchinson, Executive Director, Insect Biotech Canada, Queen's University, Kingston, Ontario, Canada K7L 3N6.**

(NW5803)A

Leicester University

RESEARCH ASSOCIATE

NEURONAL CELL SIGNALLING PROGRAMME

Department of Pharmacology & Therapeutics

This post is tenable for up to five years and is one of four research posts financed by a programme grant awarded to Professor S.R. Nahorski by The Wellcome Trust. The major research interests of this programme and related projects in this laboratory relate to the mechanisms of cell surface receptor signalling, inositol lipid hydrolysis and Ca^{2+} homeostasis in neurones. In particular, use of cells with transfected muscarinic receptor genes and investigation of phospholipases C, D and A_2 will form part of the research programme associated with this position. It is possible that part or all of this programme will eventually be located within the new IRC in Mechanisms in Human Toxicity to be established in Leicester.

The position is available from 1st January 1991 or as convenient after that date and would be suitable for someone with appropriate experience in cell biology/biochemistry/pharmacology. Previous experience with gene transfection/expression would be advantageous though not essential. Initial salary on RA 1A scale (£11,399 — £18,165) but will also include an additional enhancement premium awarded by The Wellcome Trust.

Application including a curriculum vitae, list of publications, and names and addresses of two referees should be sent by 13th October, 1990, to: **Professor S. R. Nahorski, Department of Pharmacology & Therapeutics, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, Leicester LE1 9HN.**

Informal enquiries may be made on (0533) 522923.

(4641)A

Universities of Leicester and York

POST-DOCTORAL RESEARCH ASSOCIATES

NMR Spectroscopy of Proteins: Computational Methods

As part of a collaborative programme of development of improved computational methods for use in the study of proteins by NMR spectroscopy, applications are invited for two post-doctoral positions, one based in the Biological NMR Centre, University of Leicester (Prof. G.C.K. Roberts) and one in the Department of Chemistry, University of York (Dr. R.E. Hubbard). The work will involve the development of methods (a) for the analysis of 2D and 3D NMR spectra, including the application of artifical intelligence methods to resonance assignment (in collaboration with Dr. P. Edwards, University of Aberdeen), and (b) for the calculation of structures of proteins and their complexes from NMR data. The project will provide the opportunity to gain experience of a range of modern NMR and computational techniques, working in close association with people involved in their practical application to proteins. Further information can be obtained from Prof. Roberts (0533-522978) or Dr. Hubbard (0904-432569).

The positions, funded by SERC Biotechnology Directorate, are for two years from 1st October, on salary range RA 1A (£11,399-£18,165) according to age and experience; the starting date could be delayed for an exceptional applicant. Applicants should have programming experience, and some familiarity with modern NMR spectroscopy would be a considerable advantage.

Applications, including full CV and the names of two referees, should be sent to **Professor G C K Roberts, Biological NMR Centre, University of Leicester, PO Box 138, University Road, Leicester LE1 9HN, or Dr R E Hubbard, Department of Chemistry, University of York, Heslington, York YO1 5DD.**

Closing date for receipt of applications: 20th October, 1990. (4652)A

The Queen's University of Belfast

MOLECULAR VIROLOGIST/PARASITOLOGIST

Division of Genetic Engineering. School of Biology and Biochemistry

The above post is funded by the Agricultural and Food Research Council for up to 3 years to undertake research in a new project on the possibility of using Bovine Enterovirus as a vector for carrying antigenic components of the common liver fluke, Fasciola hepatica, with the long term aim of developing a vaccine. Substantial work on the molecular biology of both these agents has already been done in the School making it possible to initiate this novel approach.

Applicants must hold an Honours Degree in a Biological Science and have some experience in genetic manipulation work. A relevant PhD and experience of immunological techniques is desirable.

Commencing salary: £11,400 per annum. Applicants, quoting ref 90/H2, should submit a curriculum vitae, including the names and addresses of two referees to the Personnel Officer at the address below. Closing date: 15th October, 1990.

POSTDOCTORAL RESEARCH ASSISTANT

Division of Biochemistry

The above post, funded by the MRC, is available immediately for 3 years to work with Dr D Allsop on the metabolism of amyloid precursor proteins in Alzheimer's disease and related disorders. Applicants must have a PhD in Biochemistry. The work will involve cell culture, immunochemical techniques including Western blotting, and the isolation and characterisation of proteins. Practical experience in one or more of these areas and a record of published work would be an advantage.

Salary scale: £11,400-£18,165 per annum, placing depending on age and experience. Applicants, quoting ref 90/I2, should submit a curriculum vitae, including the names and addresses of two referees to the Personnel Officer, The Queen's University of Belfast BT7 1NN, Northern Ireland (telephone (0232) 245133 ext 3044 or Fax (0232) 247895). Closing date: 19th October, 1990.

The University is an Equal Opportunity Employer. The University may decide to interview only those applicants who appear, from the information available, to be the most suitable in terms of experience, qualifications and other requirements of the post.

(4666)A

UNIVERSITY OF OXFORD Institute of Molecular Medicine
MRC Molecular Haematology Unit (Director: Professor D.J. Weatherall)

3 Postdoctoral Research Positions

POST 1

Applications are invited for a three year post-doctoral appointment to join a research group directed by Dr. P.C. Harris and Dr. D.R. Higgs to study the organisation and related function of DNA from the terminal chromosome band of 16p. This region contains the α-globin complex, the polycystic kidney disease I locus, and genetic loci that are responsible for some forms of mental retardation. This study is funded for 3 years by the MRC Human Genome Mapping Project and will use yeast artificial chromosomes (YACs) to clone large genomic fragments for study. Experience with molecular biological techniques is essential but training will be provided for the YAC methods.

POST II

Applications are invited for a three year post-doctoral appointment to join a research group directed by Professor D.J. Weatherall and Dr. D.R. Higgs. The aim of the project is to understand the mechanisms by which the tissue and developmental stage-specific expression of the human α-like globin genes are controlled.

Candidates for this position should have a comprehensive background in standard molecular biological techniques and previous experience in experimental approaches to the analysis of gene expression would be desirable.

POST III

Applications are invited for a three year post-doctoral appointment to join a research group directed by Dr. W.G. Wood. The aim of the project is to understand the mechanisms controlling the developmental switch in expression of the human β-like globin genes.

Candidates for this position should have a comprehensive background in standard molecular biological techniques and previous experience in experimental approaches to the analysis of gene expression would be desirable.

The salary range for each position is £11,399-£18,165 depending on experience. *The University of Oxford is an equal opportunity employer.*

For an application form and job description please contact: **The Assistant Administrator, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU. Tel: 0865 752443, quoting ref: RS1A(HWH)** and indicating the posts for which you wish to be considered. **Closing date: three weeks from the appearance of this advertisement.**

(4684)A

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY
UNIVERSITY OF OXFORD

Molecular Virology

POSTDOCTORAL RESEARCH POSITION

Salary £13,495-£18,165

Applications are invited for a one year position to study the regulation of vaccinia virus late gene expression. Applicants must posses a Ph.D. The post, funded by the Wellcome Trust, is available from 1.1.91.

Applications in writing, together with CV and names and addresses of two referees, should be sent to **The Administrator, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE by 12th October, 1990. Informal enquiries may be made to Dr. G.L. Smith (0865-275521).** (4649)A

THE ADMINISTRATOR, SOUTH PARKS ROAD, OXFORD, OX1 3RE.

HERIOT-WATT UNIVERSITY

International Centre for Brewing and Distilling

TWO POST-DOCTORAL RESEARCH ASSOCIATES

in Plant Biochemistry/Yeast Genetics/Molecular Biology

Funded by the AFRC, these posts are tenable for three years from October 1990.

One (REF 113/ 90) with Dr J H Bryce, is to investigate the regulation of seed dormancy in barley, in particular through the control of mitochondrial function at the biochemical and molecular level. Candidates should have a PhD in biochemistry or molecular biology. Salary will be in the range £11,399 to £18,165.

The second (REF 114/ 90) with Dr P G Meaden, is to identify and clone genes involved in the synthesis of beta-D-(1,3)-glucan in the cell wall of yeast (Saccharomyces cerevisiae). Candidates should have some experience of molecular biology and genetics, preferably with yeast. Starting salary will be in the range of £11,399 to £12,792.

Informal enquiries to Dr J H Bryce (031 449 5111 ext 4674) or Dr P G Meaden (ext 4694) would be welcomed.

Applications, giving details of career history, education and experience, together with the names and addresses of two referees, should be sent as soon as possible to the Staff Office, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, quoting the appropriate ref no. (4658)A

JOHN INNES INSTITUTE

SCIENTIFIC OFFICER

Required to join a group exploiting molecular techniques to study genetic variation in nitrogen fixing bacteria. The person appointed will assist in the isolation, culture and characterization of the bacteria using techniques such as RFLP, PCR and DNA sequencing. Further details from Dr P Young.

Candidates should have a degree or equivalent qualifications in an appropriate subject. The salary is £9,907 to £13,262. The post is a straight-through position with promotion to the Higher Scientific grade (salary £11,586 to £13,262) on achieving the appropriate level of experience an expertise. Non-contributory superannuation.

Applications with full particulars and CV together with the names of two referees should be sent to the **Personnel Officer, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH.**

Closing date for applications 11th October, 1990 quoting reference AG/433.

The John Innes Institute is an Equal Opportunities Employer and is associated with the AFRS Institute of Plant Science Research. (4672)A

Department of Health and Human Services
Public Health Service • National Institutes of Health
National Institute on Deafness and
Other Communication Disorders

DIRECTOR

DIVISION OF INTRAMURAL RESEARCH

Opening Date: August 17, 1990 Closing Date: October 31, 1990

(All applications must be post-marked by the closing date.)

The National Institute on Deafness and Other Communication Disorders (NIDCD) is recruiting for the position of Director, Division of Intramural Research (DIR). The Director, DIR, directs a program of multi-disciplinary fundamental and clinical research for the NIDCD. The Director, DIR, serves as the principal advisor to the Institute Director on biomedical and scientific affairs involving the intramural research programs conducted by basic and clinical research Investigators in the field of deafness and other communication disorders. The Director develops and recommends policies for the execution of the intramural research programs, determines their ongoing effectiveness, and recommends or develops new or revised direct research programs. In collaboration with senior officials, the Director, DIR, participates in the design and implementation of overall Institute management policies including program planning, budget formulation, and resource allocation. The Division of Intramural Research has an FY 1990 budget of $4.3 million and a current FTE ceiling of 24.

This is a Civil Service position in the Senior Executive Service (SES), with base salary currently from $71,200 to $79,200 per year depending on qualifications. SES members are eligible for various bonuses and awards, and physicians are eligible for a comparability allowance of up to $20,000 per year, although total compensation cannot exceed the rate for Executive Level I (currently $107,300 per year). (Alternatively, applicants may be eligible for appointment in the Commissioned Corps of the U.S. Public Health Service.) The SES individual selected, if not presently in the Senior Executive Service, must serve a one-year probationary period.

Further information about the position and qualification requirements may be obtained by contacting Ms. Christine A. Clements at (301) 402-0508 (Voice or TDD) or TDD (301) 402-1134. An Application for Federal Employment (SF-171) along with a Curriculum Vitae and Bibliography should be submitted to:

Ms. Christine A. Clements
Personnel Management Specialist
National Institutes of Health
National Institute on Deafness and
Other Communication Disorders
Building 31, Room B2C05
9000 Rockville Pike
Bethesda, Maryland 20892

NATIONAL INSTITUTES OF HEALTH

Additional information may be obtained from: Dr. Edward Korn, Chairperson, NIDCD Search Committee for Director, Division of Intramural Research, NIDCD, National Institutes of Health, Building 10, Room 7N214, 9000 Rockville Pike, Bethesda, Maryland 20892. (301) 496-2116.

NIH is an Equal Opportunity Employer

(NW5788)A

ASSISTANT PROFESSOR

MOLECULAR GENETICS

University of Illinois at Chicago

The Laboratory for Molecular Biology of the Department of Biological Sciences invites applications for a tenure track faculty position at the level of assistant professor available Fall 1991. The Laboratory is interested in further expanding its strong molecular genetics research group. Outstanding candidates with promising research programs utilizing molecular and/or genetic approaches to modern cellular or developmental problems will be considered. Successful candidates are expected to have a PhD or MD degree with postdoctoral experience, and to provide evidence that they can pursue a vigorous, independent research program, and contribute to the Department's teaching program.

Please apply before 15th November, 1990 by sending curriculum vitae, description of research interests, and names and addresses of three references to:

Molecular Genetics Search Committee
c/o Dr Sidney B Simpson, Jr, Head
Department of Biological Sciences (M/C 066)
University of Illinois at Chicago
PO Box 4348, Chicago, IL 60680

UIC is an AA/EOE

(NW5802)A

CARDIFF

DEPARTMENT OF GEOLOGY

PROJECT OFFICER/ ENGINEERING GEOLOGIST

(Fixed-Term 1 Year)

The Department seeks an Engineering Geologist with an interest in environmental issues to work as Project Officer with staff involved in Cardiff's new MSc course in Engineering Geology which commences in October 1990.

The Project Officer would be charged with developing short courses utilising permanent university staff, in environmental aspects of Engineering Geology from option units of the MSc course, aimed at industry, LEA engineers, managers and planners. He/she will also contribute to the initial year of the MSc Engineering Geology course and liaise with representatives of the construction industry and local authorities in Wales and the West.

The post is seen as either a part-time secondment, or as a full-time appointment for a suitably qualified candidate with strong industrial links.

Salary: Up to £14,038 per annum, or through part-time secondment arrangement.

For further particulars and application form please write to **University of Wales College of Cardiff, PO Box 431, Cardiff CF1 1TA** quoting Ref 90/114. Informal enquiries contact Professor Robert B Kidd or Professor Mike Brooks on (0222) 874830.

Closing date: 28th September, 1990.

(4683)A

EVOLUTIONARY ECOLOGIST

The Department of Biology at Washington University is seeking an Evolutionary Ecologist for a tenure-track faculty position at the rank of Assistant Professor. Areas of interest include, but are not limited to, ecological genetics, population and community ecology, coevolution, and behavioral ecology. The evolutionary ecologist would be expected to interact with a faculty strong in population genetics, macroevolution, systematics, and ecological genetics using molecular tools. Postdoctoral experience is preferred.

Applicants should send a curriculum vitae, statement of research and teaching interests, and three letters of recommendation by 1st December, 1990 to:

Dr Alan R Templeton, Chair
Evolutionary Ecologist Search Committee
Department of Biology
Washington University
St Louis, MO 63130

Washington University is an Equal Opportunity/Affirmative Action employer. (NW5807)A

1991-1992 visitor appointments in the High Altitude Observatory (HAO) at the National Center for Atmospheric Research

are available for up to one year in solar physics, solar-terrestrial physics, and related astrophysics. We encourage scientists with strong backgrounds in physics (experimental and theoretical) to apply. Due by 1/15/91 are: 1) vita; 2) list of publications; 3) recommendation letters from three scientists familiar with applicant's work; 4) statement of proposed research at HAO; 5) statement of financial needs, number of family members accompanying you, ages of children; 6) transcripts of graduate courses and PhD thesis abstract if less than two years' post-PhD experience.

Send to: **HAO Visitors Committee, HAO/NCAR, PO Box 3000, Boulder, CO 80307**. *NCAR is an EO/AA employer.* (NW5808)A

UNIVERSITY OF DUNDEE

Department of Surgery

RESEARCH ASSISTANT

required to investigate the role of cytoskeletal changes (particularly those in actin-associated proteins) in colorectal cancer. Experience in immunohistochemical techniques is desirable but not essential. The post is funded by a grant to Mr T C Holme and his colleagues from the Cunningham Trust for two years. Salary will be on the Research IA scale (£11,399-£12,086 pa). Informal enquiries to Dr E L Newman on (0382) 60111 ext 2551.

Applications with full CV (2 copies) and the names and addresses of two referees to The Personnel Office, The University, Dundee DD1 4HN. Closing date: 5th October, 1990. Please quote reference EST/443/90/N. (4656)A

South Oxon ANIMAL TECHNICIANS

Required to work with rodents. £7,745-£12,400 according to experience. Opportunities for further studies available. Box Number 4681, c/o Nature, 4 Little Essex Street, London WC2R 3LF.

(4681)A

NOTTINGHAM HEALTH AUTHORITY

CITY HOSPITAL

TOP GRADE (GRADE C) BIOCHEMIST

SALARY: £22,771 TO £31,164 PER ANNUM

Required to be Deputy Head of Department and share the responsibilities for providing a comprehensive analytical and advisory service to this large District General Teaching Hospital with the Consultant Chemical Pathologist.

Applicants are expected to have an appropriate higher qualification (MRCPath, MCB, PhD) and have extensive experience (within or without the Health Service).

For further information and to arrange an informal visit please contact: Dr C B Marenah, Consultant Chemical Pathologist, Clinical Chemistry Department, Telephone 0602 691169, ext. 45085.

Application forms and job descriptions from the Personnel Department, Unit Headquarters, City Hospital, Hucknall Road, Nottingham NG5 1PB. Tel: 627672 (24 hour answering service).

Closing date: 5th October, 1990.

(4635)A

POSTDOCTORAL POSITION

Available to study regulation of gene expression in retinal glial cells [see Mol. Cell. Biol. 9, 4556; DNA 8, 437 (1989)]. Ongoing studies involve characterization of transcription factors and expression of growth factors in glial cells. Experience in recombinant DNA and protein purification techniques essential. Please send curriculum vitae and names of three references to: **Vijay Sarthy, Ph.D., Department of Ophthalmology, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL 60611.** *AN EQUAL OPPORTUNITY / AFFIRMATIVE ACTION EMPLOYER.*

(NW5795)A

Molecular Geneticist/ Molecular Cell Biologist

UCSF School of Medicine

The dept. of Ophthalmology and Program in Biological Sciences is seeking a scientist who has a strong commitment to developing a research program relevant to degenerative diseases of the retina. We prefer candidates at Assistant Professor level but will consider appointment at a more senior level. Women/Minorities are encouraged to apply. EOE/AA. Send CV, summary of research accomplishments and future plans, and names of at least three references by 12th October, 1990 to: David R Copenhagen, PhD, Ophthalmology-Beckman Vision Center, University of California, San Francisco, SF, CA 94143-0730.

(NW5793)A

RESEARCH OPPORTUNITIES IN ENVIRONMENTAL MICROBIOLOGY

We are looking for a number of men and women of dedication and perception to be involved with three important new initiatives. These are concerned with the use of microorganisms to remove pollutants from the environment and the sensitive detection of those microorganisms.

You will join an established Environmental Microbiology Research Group directed by Dr R G Burns within one of the most prestigious research schools in the UK. Two of the projects will involve close collaboration with March Environmental Laboratories (a division of the March Consulting Group), a company at the forefront of environmental biotechnology R & D and based on the University of Kent campus.

1 Biotreatment of surface waters to reduce pesticide contamination (funded by the Foundation for Water Research and in collaboration with March Environmental Laboratories).

2 Anaerobic treatment of wool scouring wastes (funded by the Department of the Environment Environmental Protection Technology Programme and in collaboration with March Environmental Laboratories, UMIST and a number of companies).

3 Development of immunological techniques to detect useful microorganisms introduced into soil (funded by the Department of the Environment).

All three projects require Postdoctoral Research Fellows (initial salary £14,703) and for Projects 1 and 2 you should have experience in the use of microorganisms for pesticide degradation and associated analytical techniques. A knowledge of laboratory and pilot scale bioreactors for treating contaminated water is desirable. For Project 3 you should have experience in immunological techniques and, preferably, the development and use of monoclonal antibodies for identifying bacteria.

Projects 1 and 2 also need experienced Research Technicians (initial salary £10,297) who have a knowledge of appropriate microbiological and biochemical techniques.

Initially, appointments to Projects 1 and 2 will be for two years and to Project 3 for three years.

Interested in these challenging and rewarding positions? Then please send detailed c.v. (and the names and addresses of three referees) to Mr R Shine, The Registry, The University, Canterbury, Kent CT2 7NZ - from whom further details may be obtained - by 19 October, 1990. Informal enquiries should be made to Dr R G Burns, Canterbury (0227) 764000 Ext. 3698.

UNIVERSITY OF KENT
AT CANTERBURY
Biological Laboratory

(4659)A

UNIVERSITY OF CAPE TOWN

Dept of Microbiology:

Lecturer

Applications are invited for the above post, for appointment as soon as possible.

The post may be filled on a permanent basis or on a three year fixed-period basis, whereafter it may be renewed or converted to a permanent post.

A negotiable salary package includes attractive staff benefits such as medical aid, a pension scheme and a housing subsidy in certain circumstances.

Applications, including a full curriculum vitae, research interests and publications, present salary, and the names and addresses of three referees, should be submitted not later than 31 October 1990 to the Appointments Officer, University of Cape Town, Rondebosch 7700, Republic of South Africa; tel: (21) 650-2192; fax: (21) 650-2138. Further information (including details of salary package) may be obtained from the above, or from SASTO, 2nd Floor, 16 Charles II Street, London SW1Y 4QU.

The policy and practice of the University of Cape Town is to oppose discrimination on grounds of gender, race or creed in any sphere of university life. UCT has consistently protested, and continues to protest, against apartheid laws and practices and is committed to working towards a just future for our country. Information on this is available on request.

(W7751)A

C69686/1265-0

SCOTTISH UNIVERSITIES
RESEARCH & REACTOR
CENTRE

DIRECTOR

Following the secondment of Professor Baxter, the present Director, to the IAEA International Laboratory in Monaco, we seek to appoint a Director for three years from 1st November, 1990 or as soon as possible thereafter.

Salary will be not less than £27,000 pa.

The SURRC, with its small research reactor, was opened in 1963 to provide the Scottish Universities with central research and teaching facilities in the nuclear field. Since then, the range of activities has widened considerably, with current emphasis in the earth and environmental sciences. the Centre now has internationally recognised expertise in isotope geology and geochemistry, environmental radioactivity, radiocarbon dating, nuclear medicine and health physics, stable isotope biology, science-based archaeology, nuclear engineering, nuclear physics, radiochemistry and elemental analysis. There are substantial contacts with industry.

The Centre is currently administered by the University of Glasgow on behalf of the Universities of Glasgow, Edinburgh, Strathclyde, Aberdeen and St Andrews.

Further particulars are available from the Academic Personnel Office, University of Glasgow G12 8QQ (tel 041-339 8855 ext 5161, fax 041-330 4921), where applications (3 copies) should be lodged not later than 12th October, 1990. In reply, please quote reference number 7096(n). *The University of Glasgow is an equal opportunities employer.*

(4667)A

The University of Zurich Medical School invites applications for the position of

Professor and Head of the Institute of Virology

The applicant should have an international scientific reputation as well as an interest in clinical virology, and should represent his discipline in teaching and research.

Written applications with CV, list of publications, list of courses taught, and a summary of current and planned research are to be sent to the president of the search committee, Prof Dr A von Graevenitz, Institute of Medical Microbiology, University of Zurich, Gloriastrasse 32, CH-8028 Zurich, Switzerland.

(W7746)A

RESEARCH FELLOW

Solid State Physics

$A33,445-$A56,887 (under review)

CSIRO DIVISION OF RADIOPHYSICS
MARSFIELD, NSW, AUSTRALIA.

As a research leader with a commitment to the needs of the information and communications industries, the Division of Radiophysics engages in R&D and consulting in the areas of signal processing, solid state device design, millimetre-wave and microwave receiver technology, electromagnetics and ultrasound technology.

The Division is developing techniques for the design, fabrication and testing of Gallium Arsenide based discrete semiconductor devices and integrated circuits incorporating them. These devices have their principal application at microwave and millimetre wavelengths. Research in this area is vital to the Australian microwave semiconductor industry.

We are seeking an experienced scientist to manage a new device and MMIC processing facility to be established in the Division's clean-room facility at Chatswood. Responsibilities will include:

- Research and development aimed at developing a commercially viable device and MMIC process technology which builds on the core technology already developed within the Division.
- Close interaction and involvement with the on-going strategic research program at the Division's Marsfield laboratories.

You will need to possess the following qualifications:

- PhD degree, or equivalent qualifications, in engineering or physics
- Extensive experience in GaAs device and MMIC processing in a commercial environment
- Preferably, experience in the physics, fabrication and application of high electron mobility transistor devices.

Appointment will be for a term of three years. Australian Government superannuation benefits and, under certain conditions, assistance with living costs, are available. You are invited to telephone Dr John Archer or Mr Robert Batchelor on (02) 868 0222 or fax (02) 868 0490 for further information and copies of the selection criteria and duty statements.

Applications should be submitted, in writing, by 20 October 1990, quoting reference No. A1050. They should include full details of your professional qualifications, the names of two professional referees, and be addressed to:

The Chief,
CSIRO Division of Radiophysics,
PO Box 76, Epping, NSW 2121, Australia.

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W7748)A

DEVELOPMENTAL BIOLOGIST

Iowa State University is seeking candidates to fill an assistant/associate professor level faculty position in developmental biology. The successful candidate will be expected to establish a competitive research program and to participate in graduate and undergraduate education. Applications are encouraged from individuals interested in any area of developmental biology involving animals, plants, or unicellular eukaryotes.

The successful candidate will have space in a new molecular biology building. Generous start up funds are available. The department contains active, collaborative research groups in genetics, neurobiology, developmental biology, cell biology, molecular biology and physiology. ISU is an equal opportunity/affirmative action employer. Women and members of minority groups are encouraged to apply. Deadline for application is Dec. 17, 1990, or until the positions are filled. Candidates should send a current CV containing a description of research interests and arrange for three letters of recommendation to be sent to:

Dr. Jack R. Girton
Chair, Faculty Search Committee
Department of Zoology and Genetics
Iowa State University
Ames, Iowa 50011

(NW5811)A

An der Medizinischen Fakultät der Universität Wien ist die Planstelle eines/einer

Ordentlichen Universitätsprofessors/ Universitätsprofessorin für KLINISCHE CHEMIE UND LABORATORIUMSDIAGNOSTIK

(Nachfolge von Prof. Dr. F. Gabl)

am derzeitigen Institut für Klinische Chemie und Laboratoriumsdiagnostik bzw. am künftigen Klinischen Institut für Medizinische und Chemische Laboratoriumsdiagnostik zu besetzen.

Als Bewerber gesucht werden Fachärzte/innen für Medizinische und Chemische Laboratoriumsdiagnostik mit hervorragenden eigenständigen wissenschaftlichen Leistungen auf dem Gesamtgebiet oder einem Teilgebiet des Faches und praktischen Erfahrungen in der akademischen Lehre. Der Bewerber/in sollte zu Aufbau, Organisation und Leitung eines großen (universitären) diagnostischen Laboratoriums befähigt sein. Darüber hinaus sind Erfahrungen und Fähigkeiten zur Integration der Teilgebiete der Klinischen Chemie und Laboratoriumsdiagnostik, sowie die Bereitschaft zur aktiven Zusammenarbeit mit den Kliniken, notwendig.

Bewerber haben das Fach Klinische Chemie und Laboratoriumsdiagnostik in Forschung und Lehre zu vertreten, sowie das Zentral-Laboratorium des Universitäts-Klinikum zu leiten.

Eine Beschreibung der derzeit gegebenen räumlichen und personellen Voraussetzungen, sowie der künftigen Aufgaben, Verhältnisse und Strukturen des Instituts für Klinische Chemie und Laboratoriumsdiagnostik (Klinisches Institut für Medizinische und Chemische Laboratoriumsdiagnostik) im Neuen Universitäts-Klinikum mit etwa 2000 Betten kann vom Dekanat der Medizinischen Fakultät der Universität Wien angefordert werden.

Folgende Unterlagen sind dem Dekanat der Medizinischen Fakultät der Univiersität Wien einzureichen:

1) Lebenslauf und wissenschaftlicher Werdegang
2) Eine der Verwendung entsprechende abgeschlossene inländische oder gleichwertige ausländische Hochschulbildung (Doktorat der gesamten Heilkunde), sowie den Nachweis der Anerkennung als Facharzt für Medizinische und Chemische Laboratoriumsdiagnostik bzw. einer entsprechenden Qualifikation.
3) Eine an einer österreichischen Universität erworbene oder gleichwertige ausländische Lehrbefugnis (venia docendi) oder eine der Lehrbefugnis als Universitätsdozent gleichzuwertende wissenschaftliche Befähigung für das Fach, das der zu besetzenden Planstelle entspricht.
4) der Nachweis der pädagogischen Eignung
5) Liste aller wissenschaftlichen Veröffentlichungen, sowie zehn vom Bewerber als wesentlich eingestufte Arbeiten in Form von Sonderdrucken und gegebenenfalls Aufstellung abgehaltener Lehrveranstaltungen und
6) Nachweis der angeführten Qualifikation.

Bewerbungen sind bis zum 31. Oktober 1990 an das Dekanat der Medizinischen Fakultät der Universität Wien. A-1010 Wien Dr. Karl Lueger Ring 1, zu richten.

(W7757)A

HEWLETT PACKARD BIOCHEMIST

HPLC APPLICATIONS CHEMIST

Hewlett-Packard, a world leader in HPLC instrumentation, seeks a biochemist to join the applications group at its HPLC manufacturing division located in the northern Black Forest area of Germany.

The responsibilities for this position include developing and publishing applications based on HPLC separation of biological molecules, support of product marketing and R&D, and presentation of technical seminars and training courses.

Candidates should have a Ph.D. plus 1-2 years industry or research experience in HPLC separations of proteins, peptides, amino acids, or nucleic acids. Experience in electrophoresis and other analytical biochemistry techniques is very desirable. Strong communication skills are essential, with fluency in English, both written and oral.

Hewlett-Packard offers a flexible work environment with opportunity to interact with a diverse and talented team around the world. Applicants should send their resume (Reference No. W93) to Roland Baur, Personnel Manager, Hewlett-Packard Strasse, D-7517 Waldbronn, Germany.

(W7742)A

THE ROYAL SOCIETY

NAPIER RESEARCH PROFESSORSHIP

The Royal Society invites applications for the Napier Research Professorship, tenable from 1 October 1991, for researcn into the causes of cancer including any corresponding or allied disease and the means of its prevention, cure and alleviation. The salary will be on the prevailing UFC professorial minimum plus 40% and include superannuation. The Napier Research Professor may bid for annual research expenses of £13 500 or higher and for support for [illegible] research or technical assistant, and [illegible]ere will be a start-up grant of up to [illegible]5 000. A successful applicant from [illegible]erseas will receive assistance with removal expenses.

The appointment must be held in an appropriate higher educational or research institution in the UK. There is no restriction as to citizenship of candidates. The tenure is for five years in the first instance and is then renewable up to age 65.

Application details are available from Mrs U.M.A. Tokle, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG (Fax 071-930 2170). The closing date for applications is 16 November 1990; no applications arriving after 4 p.m. on that date will be considered.

(4520)A

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nature
— the professionals' choice

University of Dundee

Department of Surgery

POSTDOCTORAL SCIENTIST

required to work on coniugates between photosensitizers and growth factors as potential anti-tumour agents. The project is funded by the Cancer Research Campaign for three years.

Salary on the Research IA Scale (£11,399-£14,744 pa). Previous experience in one or more of cell culture, HPLC and peptide chemistry would be advantageous. Informal enquiries to Dr E L Newman on (0382) 60111 ext 2551.

Applications with full CV (2 copies) and the names and addresses of two referees to **The Personnel Office, The University, Dundee DD1 4HN.** Closing date: 5th October, 1990. Please quote reference EST/444/90/N.

(4657)A

POSTDOCTORAL POSITION

Available for Ph.D. with 1-2 years postdoctoral experience in behavioral neuroscience. Individual should have experience working with rodent models of stress using behavioral, anatomical, and neurochemical methods. Active programs include: CRF modulation of behavior, neurochemical subtrates of defensive responses; and models of anxiety and depression. Send CV and names of references to: **Ned H. Kalin M.D., Dept of Psychiatry, B6/268, University of Wisconsin, 600 Highland Ave., Madison, WI 53792.** *The University of Wisconsin is an Affirmative Action/Equal Opportunity employer.* (NW5796)A

TENURED AND TENURE-TRACK ASTRONOMERS

The Space Telescope Science Institute (ST ScI) has several openings for tenured or tenure-track astronomers. Excellence in research is the primary qualification for these positions. The level of the appointments — assistant, associate, or full astronomer — will be determined by the experience and accomplishments of the successful applicants. Tenured and tenure-track astronomers at SI ScI spend about 50% of their time in personal research and the remainder in support of the science mission of the Hubble Space Telescope. There is some latitude within this framework to tailor the functional component of the positions to the interests and expertise of the successful applicants.

ST ScI supports the research of its scientific staff through visitor and post-doctoral programs. Several workshops and symposia are held each year at the Institute. Internal funds are available for computing and other equipment. Members of the staff may obtain additional support from any of the regular NASA grant programs. The salaries and benefits are commensurate with those at AURA member universities (without the need to apply for summer support).

Candidates for these positions must have a Ph.D. or equivalent degree in Astronomy or Physics and some post-doctoral experience. ST ScI is committed to equal opportunity; women and minorities are strongly urged to apply. Candidates should send a curriculum vitae and bibliography, a brief statement of research and functional interests, and the names of four references to the Personnel Manager, Space Telescope Science Institute, 3700 San Martin Drive, Baltimore, Maryland 21218. Applications will be accepted until 31 December 1990.

SPACE
TELESCOPE
SCIENCE
INSTITUTE

An Equal Opportunity/Affirmative Action Employer, M/F/H/V (NW5815)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

DEPARTMENT OF VIROLOGY

POST-DOCTORAL FELLOW

A vacancy exists for an enthusiastic Post-Doctoral Fellow to join a group actively involved in research on cytomegalovirus and its interaction with the host immune system. This is a 2-year Project on the immunologic detection of cytomegalovirus in body fluids, where it is associated with the host protein beta2-microglobulin. Previous experience in monoclonal antibody production, immunoassays, or characterisation or viral proteins desirable.

Full job description available from the School Office, RHFSM, Rowland Hill Street, London NW3 2PF (Tel: 071 794 0500 ext 4262) to which applications (3 copies of curriculum vitae with names and addresses of 2 referees) should be sent by 4th October, 1990. Please quote reference: VIR/PD/135. (4647)A

RESEARCH SCIENTISTS

A wholly US-owned company involved in the manufacturing of diagnostic products and research & development in monoclonal antibodies and related research, seeks qualified applicants to work on the development of human monoclonal antibodies.

The candidate should have a PhD with some postdoctoral experience in the following areas:
1. monoclonal antibody production
2. cloning and expression of mammalian genes

We offer excellent remuneration and benefits package.

Interested applicants should send their curriculum vitae, publications list and names of three referees to:

Director of R&D
SCITECH GENETICS PTE LTD
12 Science Park Drive #04-04
The Mendel
Singapore 0511

(W7743)A

CLINICAL GENETICIST/ EPIDEMIOLOGIST

NATIONAL CANCER INSTITUTE

The National Cancer Institute, National Institutes of Health, is seeking applicants for Chief, Clinical Genetics Section, Clinical Epidemiology Branch, Epidemiology and Biostatistics Program. This scientist would head an intramural group which initiates research to provide new insights into the origins of human cancer. The section investigates peculiarities of cancer occurrence through the disciplines of clinical medicine and epidemiology and collaborations with cellular and molecular geneticists. Current emphasis is given to the clinical delineation of cancer family syndromes and disorders that predispose to cancer such as the neurofibromatoses and some birth defects, to gene mapping studies, and to studies of the late effects of cancer treatments including reproductive toxicity. Liaison is maintained with many clinical services throughout the Washington area and beyond, as well as with the NIH Interinstitute Medical Genetics Program which is accredited by the American Board of Medical Genetics.

Applicants should have a M.D. or Ph.D. or equivalent training in clinical genetics, epidemiology or a related field and be devoted to conducting studies related to cancer etiology. The type of appointment and salary are dependent on experience and qualifications. Starting salary ranges from $50,000-70,000. In addition to the base pay, the M.D. candidate may be eligible for a physician's comparability allowance of up to $15,000 depending on service and qualifications. Applicants should send their curriculum vitae and bibliography (or requests for further information) to:

Dr. Dilys M. Parry
Clinical Epidemiology Branch
Division of Cancer Etiology
National Cancer Institute
Executive Plaza North
Room 400
Bethesda, Maryland 20892
(301) 496-4947

NATIONAL INSTITUTES OF HEALTH

U. S. Citizenship required
An Equal Opportunity Employer

(NW5804)A

Yale UNIVERSITY

Plant Biologist

The Department of Biology at Yale University wishes to appoint ladder faculty at either the junior or senior level with broad interests and experience with plants. We seek applicants who are using a variety of approaches to study fundamental problems in plant development, cell and molecular biology, and physiology, and who can contribute to undergraduate and graduate teaching.

Please send curriculum vitae, description of research interests and the names and addresses of at least three referees by January 1, 1991 to: **Plant Biologist Search Committee, c/o Chairman, Department of Biology, Yale University, P.O. Box 6666, New Haven, CT 06511-8112.**

Yale University is an Equal Opportunity/ Affirmative Action Employer

Women and members of minority groups are especially urged to apply. (NW5789)A

FACULTY POSITION

Department of Biochemistry

Stanford University School of Medicine

Application or nominations are invited for a faculty position in The Department of Biochemistry at the level of Assistant Professor.

Stanford University is committed to increasing representation of women and member of minority groups on its faculty and particularly encourages applications from such candidates.

Applicants should have an established record of excellence in original research and in teaching. Candidates should submit a *curriculum vitae* including a list of publications, a description of their research interests, and names, address and telephone numbers of three references to **Search Committee Chairman, Department of Biochemistry, Beckman Center, Stanford University School of Medicine, Stanford, CA 94305-5307.**

(NW5817)A

THE UNIVERSITY OF NEW
SOUTH WALES
SYNDEY, AUSTRALIA

SCHOOL OF
BIOCHEMISTRY

LECTURESHIP IN
GENETICS (REF 672)

Applications are invited for
above post which will be availab
from 1st March, 1991. Appoin
ment will either be with tenure or
the basis of a contract with provisi
for conversion to tenure.

Applicants with experience in a
area of Genetics are welcome
though preference will be given
those with a strong background
an aspect of Molecular Genetics.

Salary in the Lecturer rang
A$33,163 to A$43,096 per annu

Further information from Prof
sor I W Dawes, Professor of Gen
tics, School of Biochemistry on (
ternational) 61 2 697-2089; Fax:
2 662-2918. Submit written app
cation to: The Recruitment Offic
Staff Office, PO Box 1, Kensingt
NSW 2033, Australia.

Applications close 16th Nove
ber, 1990. (W7749)

THE ROYAL SOCIETY

ROYAL SOCIETY RESEAR
PROFESSORSHIPS

The Royal Society inv
applications for four Royal Soc
Research Professorships tena
from 1 October 1991 for researc
the physical sciences (inclu
mathematics, engineering
technology). Salaries will be
above the prevailing professo
minimum and inc
superannuation. Rese
professors may bid for an
research expenses of £13 500
higher and for support for rese
or technical assistants, and there
be start-up grants of up to £35
Assistance will be given
removal expenses for succe
applicants from overseas.

Appointments, which are ten
from 1 October 1991 and are
only to British citizens, are nor
held at universities in the U
Kingdom, and are for five yea
the first instance. Details of te
will be decided later but
expected that one of the
vacancies will be renewable unt
65 and the other three for
further five-year period after
initial five years.

Application details are avai
from Mrs U.M.A. Tokle, The R
Society, 6 Carlton House Ter
London SW1Y 5AG. (Fax 071
2170). The closing date
applications is 16 November
no applications arriving after 4
on that date will be considered.
(452

UNIVERSITY OF BRISTOL
Bristol Arthritis Research Group

Applications are invited from suitably qualified candidates for the post of

RESEARCH ASSISTANT
(Research Grade 1B, £11,399-£13,495)

The successful candidate will develop immunoassays to follow the progression of osteoarthritis. A background in biochemistry/immunology would be an advantage. The appointment will be for five years and is funded by a joint ARC programme to members of the Group.

Informal enquiries may be made to Dr C J Elson on 0272 303441.

For further details telephone Bristol 303136 (ansaphone after 5.00pm) or write to the Personnel Office, Senate House, Bristol BS8 1TH. Please quote reference 430.

AN EQUAL OPPORTUNITIES EMPLOYER (4651)A

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FACULTY POSITION

Nucleic Acid Enzymology. The Department of Biochemistry at the Albert Einstein College of Medicine is seeking a tenure track **Assistant Professor** interested in the enzymology of nucleic acid metabolism. Structure or mechanism of enzymes of nucleic acid replication, transcription, degradation, covalent modification or processing are of interest. Related areas are also of interest.

Applicants should send a current curriculum vitae. The names of at least three referees, a summary of ongoing research interests and a brief proposal of future research plans. Submit applications to **Dr. Julius Marmur, Department of Biochemistry, Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, 1300 Morris Park Avenue, Bronx, NY.** (NW5813)A

MEDICAL RESEARCH COUNCIL
Anatomical Neuropharmacology Unit
Oxford University Department of Pharmacology
LABORATORY TECHNICIAN

Applications are invited for the post of Laboratory Technician Grade D, to assist in an international research programme on the brain. The work involves light and electron microscopical analysis of the molecular composition of the nervous system. Qualifications required: 'O' levels and 'A' levels in relevant subjects (or equivalent) and appropriate technical training. Salary scale: £9,706-£11,589 pa. Previous laboratory experience is essential, experience in histology would be an advantage. University terms and conditions of service apply, including 40 days of paid holidays.

Applications, together with curriculum vitae (telephone number incl) and names of two referees, should be forwarded as soon as possible to: Dr P Somogyi, Associate Director, MRC Anatomical Neuropharmacology Unit, University Department of Pharmacology, South Parks Road, Oxford OX1 3QT. Tel: Oxford 275181. (4693)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

Department of Pharmacology and Clinical Pharmacology

RESEARCH ASSISTANT
Electrophysiologist for work on Single Smooth Muscle Cells

An experienced electrophysiologist with a special interest in working with single smooth muscle cells is required. The post is supported for 3 years by an MRC Programme Grant to Professor T B Bolton. Salary up to £19,932 per annum including London Allowance.

For further information contact Professor T B Bolton on 081-672 9944 ext 55617. Further details and an application form from the Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, 081 784 2791 (24-hr answerphone). Closing date: 15th October, 1990. Please quote reference 112/90. (4678)A

UNIVERSITY OF BRISTOL
Medical School
Department of Pathology and Microbiology
A Post-Doctoral Research Associate

is required by the Cancer Research Campaign-funded Epstein-Barr virus group for a period of three years starting as soon as possible. The successful applicant will work on Epstein-Barr virus gene expression in tumours associated with the virus and their possible role in tumour induction. He/she will also be responsible for the expression of other viral genes in other systems as part of an Epstein-Barr virus vaccine development program. A background in molecular virology would be ideal although applicants with experience in other areas of molecular biology will be considered. The Department has several strong and well funded groups working on molecular and cellular aspects of cancer.

Informal enquiries can be made to Dr Andrew J Morgan on Bristol (0272) 303451.

For further details telephone Bristol 303136 (ansaphone after 5.00pm) or write to the Personnel Office, Senate House, Bristol, BS8 1TH. Please quote reference 431.

An Equal Opportunities Employer. (4682)A

POSTDOCTORAL POSITION IN POPULATION BIOLOGY
UNIVERSITY OF BASLE, SWITZERLAND

For one year, salary ca 60,000 Swiss francs, PhD and spoken German. To collaborate on selection experiments on the evolution of reaction norms in fruitflies. The ideal candidate has experience with *Drosophila* and is familiar with quantitative or population genetics and life history evolution. If all goes well, the successful applicant will be a leading candidate for a 5 year Assistant position with teaching responsibilities and possibilities for Habilitation.

Send CV and names and addresses of two referees to Prof S C Stearns, Zoology Institute, Rheinsprung 9, CH-4051 Basel, Switzerland. (W7753)A

POSTDOCTORAL/ ASSISTANT PROFESSOR
(non-tenure track)

Research appointments are occasionally available in the Department of Biochemistry at Emory University School of Medicine, Atlanta, GA 30322. Appointments are normally made on a year-by-year basis. A description of the research interests of the faculty is available from the department upon request, and highly qualified persons are invited to apply directly to the faculty member with whom they wish to work. If no position is available with the faculty member, the application may be circulated to others in the department with similar research interests.

Applications should include a resumé, a brief statement of research interests and goals, and the names and addresses of three references.

Emory University is an Equal Opportunity/Affirmative Action Employer. (NW5814)A

QUEEN'S UNIVERSITY
Kingston, Ontario, Canada

RESEARCH BIOCHEMIST, MOLECULAR/CELLULAR BIOLOGISTS, PhD

Queen's University, assisted by a new funding initiative from the Heart & Stroke Foundation of Ontario, is planning to substantially expand its existing program in hemostasis/thrombosis in the Faculty of Medicine. One, and possibly two, positions are available. The overall theme of the program is "The Regulation of Hemostasis in Health and Disease". The activities of the program currently range from the use of carefully characterized whole animal models to the analytical biochemistry of the hemostatic system. We now wish to recruit basic scientists with interests in the biochemistry and/or molecular/cellular biologic aspects of the theme of the program. The Faculty of Medicine is currently committed to developing areas of excellence and providing integration of intellectual and physical resources.

The primary appointment will be in the Department of Pathology, although cross-appointments in other departments, for example Biochemistry, will be available for appropriately qualified candidates. Although primarily research in nature, some participation in the teaching program will be expected in the area appropriate to the applicant's expertise. The positions will likely be at the Assistant Professorship level but this is negotiable depending on previous experience.

It is expected that at least one of the applicants will establish independent research activities relating to the biochemistry and/or molecular/cellular biology of fibrinolysis. The successful applicants should have demonstrated a high level of productivity and potential for establishing an independent program of research in their chosen area of activity. Major emphasis will be placed on evidence that an individual's area of interest will integrate with existing programmatic activities.

Candidates of either sex are equally encouraged to apply.

Applications with curriculum vitae or enquiries should be directed no later than 30th November, 1990 to: **Dr Alan R Giles, Department of Pathology, Richardson Laboratory, Queen's University, Kingston, Ontario, Canada K7L 3N6. Telephone (613) 545-6342.** (NW5805)A

UNIVERSITY OF READING
Department of Biochemistry & Physiology
School of Animal and Microbial Sciences

POST DOCTORAL CELL/ MOLECULAR BIOLOGIST

A post doctoral position funded by the Wellcome Trust is available for up to 3 years in the Molecular Endocrinology Laboratory. The purpose of the project is to study the post-translational processing and intracellular compartmentalization of human corticotrophin releasing hormone in stably transfected cell lines. The project will involve the use of cell culture, cell transfection and recombinant DNA technology and experience in one or more of these techniques is desirable. Starting salary will be up to £16,755 pa on the RA1A scale.

Informal enquiries to Professor P J Lowry, Tel. (0734) 318014. Apply for application form to Personnel Officer, University of Reading, Whiteknights, PO Box 217, Reading RG6 2AH, tel. (0734) 318754. Please quote Ref: R9039. (4660)A

UNIVERSITY OF ABERDEEN
Department of Agriculture

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the post of Postdoctoral Research Assistant to study the effects of environmental factors (water availability, pH, oxygen tension and microbial competition) on the growth and survival of listeria in silage.

Applicants should have experience in mocrobial physiology and a keen interest in the microbiology of food/feedstuff preservation. The post is tenable for 3 years with starting salary on the IA scale.

Informal enquiries to Dr Fenlon (0224 480291). Applications by letter and CV (two copies) to the Personnel Department, University of Aberdeen, Regent Walk, Aberdeen AB9 1FX by 19th October, 1990, quoting reference LG/048. (4690)A

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Tokyo	Tel: (03) 267 8751	Fax: (03) 267-8746
Munich	Tel: (089) 52 40 82	Fax: (089) 5 23 22 22
Paris	Tel: (1) 48 87 24 43	Fax: (1) 48 87 46 71

POSTDOCTORAL FELLOWSHIP IN DEVELOPMENTAL ASPECTS OF LOCOMOTION

A position is available immediately for a postdoctoral candidate interested in working in the general area of developmental aspects of locomotion in normal children and children with cerebral palsy. Experience in human motor system research, writing data acquisition and analysis programs as well as a background in kinematic and dynamic analysis are highly recommended.

Send curriculum vitae, statement of research interests and list of three references to: Dr James R Bloedel, Division of Neurobiology, Barrow Neurological Institute, 350 West Thomas Road, Phoenix, Arizona 85013.

An Equal Opportunity/Affirmative Action Employer. (NW5792)E

POSTDOCTORAL FELLOWSHIP/DIABETES

Research available immediately to study insulin action and insulin receptor regulation. Particular emphasis on role of tyrosine phosphatase, vanadate. Ph.D. or M.D. Experience in cell culture, molecular biology/tyrosine kinases desirable. Salary per MRC Canada.

Send CV, summary of previous training and research goals and names of three references to: Dr. I. George Fantus, Polypeptide Hormone Laboratory, Room 211, Strathcona Medical Building, McGill University, Montreal, Quebec H3A 2B2, Canada. (NW5786)E

The Wellcome Trust

Wellcome Travelling Research Fellowships 1990 - 1992

Applications are invited from post-doctoral workers to undertake research projects in any branch of the natural and clinical sciences which has a bearing on human or animal medicine (except cancer). Two fellowship schemes are available.

TO CONTINENTAL EUROPE

Tenable at academic institutes in Europe and the Soviet Union.
Eligible persons must have been engaged in research in the UK or Ireland for the last three years.
Duration: three months to two years.
Closing Dates: for full applications 23rd November 1990 and 1st May 1991. (Short-term fellows (less than one year) may apply at any time.)

TO THE UNITED KINGDOM AND IRELAND

Tenable at academic institutes in the UK and Ireland.
Eligible persons should be European, or Soviet nationals, and have a position to return to in the country from which they apply.
Duration: three months to one year.
Closing Dates: none, available all year.

Nordic candidates should apply in the first instance to: The Carlsberg Foundation in Denmark, the Sigrid Juselius Foundation in Finland, the Anders Jahre Foundation in Oslo or the Norwegian Research Council for the Sciences and the Humanities, or the Swedish Medical Research Council.

Requests for application forms should include a brief curriculum vitae, an outline of the proposed research (two pages), and a supporting letter from a full-time staff member of the host Institute. Letters to be sent quoting reference: N to: The Grants Section (Europe), The Wellcome Trust, 1 Park Square West, London NW1 4LJ.

(4644)E

UNIVERSITY OF SURREY
Department of Biochemistry
AFRC POSTDOCTORAL RESEARCH FELLOWSHIP
PROTEIN-PROTEIN INTERACTIONS

Applications are invited for a three-year AFRC postdoctoral Research Fellowship to study the molecular basis for interactions of proteins in gelation which influences the texture of food. The project will pioneer an exciting range of techniques in the interactions field such as NMR, immunocytochemistry, molecular modelling and chromatography with radioisotopes in collaboration with experts in major UK institutions and within the University. Candidates with a PhD and experience in Biochemistry, Molecular Science, Food Science/Chemistry or a relevant area should send a CV and the names of two referees to **Dr N K Howell, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH (Tel: 0483 571281 ext 2523).**

Candidates without a PhD, but with extensive postgraduate experience will also be considered. 4675E

THE UNIVERSITY OF BIRMINGHAM
School of Biochemistry
RESEARCH FELLOWSHIP

'Organisation of FNR-dependent promotors and regulation by multiple transcription activator proteins'.

Tenable for 3 years from 1st January, 1991. The project will be appropriate for a newly-qualified PhD with experience in molecular biology, molecular genetics or biochemistry. Adaptable microbiologists or chemists or graduates with relevant research experience but no PhD are also encouraged to apply.

Salary on a range £11,399-£18,165.

Informal enquiries to Dr J A Cole 021 414 5440.

Application forms (returnable by 15th October, 1990) and further particulars available from the Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT. Tel: 021 414 6483 (24 hours). Ref: S13069.

The University is an equal opportunities empployer. (4665)E

RESEARCH FELLOWSHIP
GIRTON COLLEGE CAMBRIDGE

Applications are invited for a Research Fellowship open to graduates of any university for research in a scientific subject. The Fellowship is tenable for three years from 1st October, 1991. The applications should be in the fields of Mathematics, Natural Sciences, Geography and allied subjects.

The emolument of the Fellowship is reviewed annually. From 1st October, 1990 the stipend for a person who has not completed a Ph.D is £7535 per annum; for a person with a Ph.D, £9900 per annum rising by two increments to £10100 per annum. Financial support from other sources may be taken into account.

The Fellowship, at the wish of the Fellow, may be pensionable under USS. Residence is provided, if required; a charge of £93 per month is made towards residence, and Commons (i.e. meals) are free of charge. If the Research Fellow is married, and lives out, there is a living out allowance of £1116 p.a. A child allowance of £250 per annum for the first child is made. Research Fellows may, if they wish, be permitted to teach up to six hours per week. Research expenses of £1200 over the three years may be claimed for approved Applications (5 copies) on a form which may be obtained from the **Secretary to the Electors, Girton College, Cambridge CB3 0JG, must be returned to him not later than 24th October, 1990.** A statement of approximately 1000 words outling the work candidates wish to submit in support of their applications and the research they propose to do if elected, will be required at the same time as the application is returned. The applicant should also include a short resume of his/her research for an informed layman. Again 5 copies of each of these should be included with the application. If the applicant is short-listed he/she will be asked to submit work in support of the application immediately after 30th October, 1990; it is suggested that the work to be submitted should be a dissertation and/or other writings. If the work in support of the application has not been received within 14 days, the Electors will not consider the application further. Since the College is advertising for a Research Fellow in the Arts at the same time Science applicants should state when writing for an application form whether their subject of research is an Arts or a Science subject. (4636)E

POST-DOCTORAL FELLOWSHIP OBSERVATIONAL ASTROPHYSICS

A post-doctoral fellowship is available at the Space Telescope Science Institute to support ongoing research involving the interpretation of observations of accretion processes in cataclysmic variable stars related systems. The position is funded by grants to K. Horne, and will initially involve work on tomographic imaging or accretion disks and may later include analysis and interpretation of time-recovered Faint Object Spectrograph observations from the Hubble Space Telescope. The fellow will have full access to the research facilities at ST ScI.

Candidates must hold a Ph.D. degree in Astronomy or Physics, have strong computer skills and relevant experience which ideally will include analysis of time-resolved spectroscopic observations. The initial appointment for one year may begin at any time. Renewal for a second or third year is contingent upon continued funding. Applications, including a curriculum vitae, a statement of research interests, a list of publications, and three letters of recommendation, should be sent to the Personnel Manager at ST ScI. The position may be filled whenever a suitable candidate has been located. Minorities and females are strongly urged to apply.

Space Telescope
Science Institute
Personnel Office
3700 San Martin Drive
Baltimore, MD 21218

An Equal Employment Opportunity, Affirmative Action Employer
M/F/H/V (NW5816)E

ARC POST-DOCTORAL RESEARCH FELLOWSHIPS

The Arthritis & Rheumatism Council has introduced a new post-doctoral fellowship scheme as part of a staged career structure to attract and retain talented scientists in rheumatological research. Applicants should normally be within their first or second post-doctoral appointment (although there is no age limit) and should be associated with established researchers/sponsors, preferably within a multidisciplinary research group.

These fellowships will be awarded for 5 years in the first instance and fellows who are making significant research contributions may be considered for 3-year renewals subject to satisfactory scientific review. Salary will usually be within the range IA or II (plus the ARC enhancement premium) and application may be made for reasonable running costs.

Applications in quadruplicate on the appropriate form must be submitted jointly by the candidate and the sponsor and should outline the proposed programme of research. Application forms are available from the **Research & Education Department, ARC, 41 Eagle Street, London WC1R 4AR.** Completed application forms must be sent to the Research & Education Secretary to arrive **Monday, 5th November, 1990.**

(4654)E

UNIVERSITY OF CAMBRIDGE
Postdoctoral research fellowship
Molecular biology of glial cells

This is a three year fellowship, funded by the MRC. The project is to use recombinant DNA and ribozyme technology to alter the expression of genes for cell surface adhesion molecules, proteases and protease inhibitors in glial cell lines, and assay for effects on *in vitro* and *in vivo* models of axon regeneration. Candidates should be trained in molecular biological techniques.

For further information call J. Rogers or J. Fawcett on (0223) 333899.

Send written applications, including c.v. and the names of 2 referees to:- **Dr. J. Rogers, Physiological Laboratory, Downing St., Cambridge CB2 3EG, England.** (4643)E

THE UNIVERSITYOF BIRMINGHAM
Superconductivity Research Group
SCHOOL OF PHYSICS AND SPACE RESEARCH
RESEARCH FELLOWSHIPS

Two postdoctoral appointments for research on HTC Superconductors in the Birmingham Interdisciplinary Superconductivity Research Group. These posts are supported by the SERC on a 4 year rolling grant. Familiarity with the physical properties of HTC superconductivity, vacuum technology or instrumentation would be preferred. Applicants should have or be about to obtain a PhD in Physics or a related Engineering subject.

Salary on a range £11,399-£18,165.

Application forms (returnabled by 9th October, 1990) and further particulars available from the Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT. Tel: 021 414 6483 (24 hours). Ref: S13094.

The University is an equal opportunities employer. (4664)E

AFRICAN MONTANE ECOLOGY RESEARCH FUNDS AVAILABLE NOW

$30,000 per year available for basic and applied field research on the ecology of African montane ecosystems. Applications welcomed from qualified agriculturalists, botanists, climatologists, ecologists, geographers, geologists, zoologists etc. Applicants should have at least the equivalent of a bachelor's degree. Grants tenable at Karisoka Research Centre in Virunga Volcano region of Rwanda. For further details, inquire with stamped, addressed envelope to: AME Programme, "The Digit Fund", 45 Inverness Drive, East, Englewood, Colorado 80112, USA.

(NW5809)N

STUDENTSHIPS

University of Göteborg (Gothenburg) Sweden

Theoretical Physical Chemistry

Research Fellowship — Studentships

— paying approx $20,000 (US) per annum to support a full-time PhD student for up to 4 years, working towards a PhD degree in Physical Chemistry. The research will be based on statistical mechanics and molecular dynamics simulation applied to internal molecular energy transfer and chemical reaction rates. Requirements: Proficiency in English; an undergraduate degree in Chemistry or Chemical Physics.

The position is open for application until 29th October, 1990. Send your application including a verified transcript of your academic record and addresses of two academic staff members willing to write letters of reference to:

Registrator, Universitetsförvaltningen, Vasaparken,
S-411 24 Göteborg, Sweden.

Refer to the reference number "E 334 2460/90" in your application.

Further information can be obtained from:

Professor Sture Nordholm
Department of Physical Chemistry
University of Göteborg/CTH
S-412 96 Göteborg, Sweden.
Phone: +46-31-722831; Fax: +46-31-167194.

(W7754)F

UNIVERSITY OF DURHAM

Department of Biological Sciences

PhD Studentship in Plant Molecular Biology

An SERC research studentship is available to investigate tissue-specific gene expression during flower development in Brassica species. The successful applicant will join an active research group working on various aspects of plant molecular biology.

Applicants should have a good honours degree in a biological science with a strong interest towards research in molecular biology.

Applications, including a CV and the names of two referees, should be submitted by 12th October, 1990 to: Dr R Croy, Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE (Tel: 091-374 2433).

(4650)F

ASSISTANTSHIPS

MARIE CURIE CANCER RESEARCH

RESEARCH ASSISTANTSHIP IN GENE REGULATION

Applications are invited for the post of Research Assistant in the laboratory of Dr Peter O'Hare to take part in a programme of work on the interaction of host and virus regulatory proteins in the control of herpesvirus transcription and on the identification of novel antiviral compounds which may specifically interfere with virus gene expression. While no formal experience in transcriptional studies or protein purification is required, applicants should have some practical experience of gene cloning and have an undergraduate degree in a relevant subject. Competitive salary will depend upon age, qualifications and experience.

Please submit application, including a curriculum vitae and the names and addresses of two referees, to **Dr Peter O'Hare, Marie Curie Research Insitute, The Chart, Oxted, RH8 0TL, Surrey. (Telephone No: 0883-722306).**

(4633)P

GRANTS & SCHOLARSHIPS

THE ROYAL SOCIETY RESEARCH GRANTS SCHEME

Grants of up to £10 000 are available from the Society to academic workers in UK higher education establishments for research projects in the natural sciences, including mathematics, engineering science, agricultural and medical research. These grants are intended to meet, primarily, the cost of specialized equipment and essential consumable materials, but may also cover travel and subsistence costs for essential field research visits in the UK.

The next closing date for receipt of applications is **15 November 1990.** Full details and application forms may be obtained from the Executive Secretary (Ref: JECL), The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG (071-839 5561, ext. 220).

(4640)H

COURSES & CONFERENCES

NATURE CONFERENCE

NEW HORIZONS IN IMMUNOLOGY

15-16 November 1990
Boston,
at the Copley Plaza Hotel

CONFERENCE SESSIONS

1. TOLERANCE AND THE IMMUNE REPERTOIRE
2. ANTIGEN PROCESSING AND PRESENTATION
3. AUTOIMMUNITY AND CONTROL OF IMMUNE RESPONSE
4. VACCINES AND ANTIBODIES

REGISTRATION FEES

Standard rate including reception: $350

Special rates for full-time academics and government employees only: $300

Contact: Diana Berger, Nature Publishing Company,
65 Bleeker Street, New York, NY 10012-2467 USA.
(Tel: 212-477-9600)

(NW5629)C

dkfz

GENIUS

COMPUTER ANWENDUNGEN FÜR DEUTSCHE BIOLOGEN UND MEDIZINER

Ab sofort is GENIUS (Genetisches Interaktives Unix System) am Deutschen Krebsforschungszentrum in Heidelberg verfügbar.

Molekularbiologen und Humangenetiker in ganz Deutscland haben damit die Möglichkeit, von ihren lokalen Arbeitsplätzen aus auf dem GENIUS-Computer in Heidelberg zu arbeiten. Die Verbindung zu dem Computer ist über alle wichtigen deutschen Datennetze (WIN, Datex-P) möglich.

GENIUS kombiniert die Rechenleistung eines Hochleistungs-Computers (Convex C210) mit der Verfügbarkeit aller bedeutenden Protein- und DNA-Datenbanken (der jeweils aktuellsten Versionen) sowie einer Sammlung leistungsfähiger Programme für Sequenzanalyse und ver-wandte Problemstellungen).

GENIUS bietet u.a. folgende Dienste:

— **EMBnet-Knoten:**
— Zugriff auf täglich neueste EMBL (bzw. Genbank)-Sequenzdaten.
— Anschluß an das EMBnet Computer-Mail System.

— **HUSAR** (Heidelberg Unix Sequence Analysis Resources):
Unix-Programme zur Sequenzanalyse, die kompatibel zu dem GCG-Paket der University of Wisconsin sind. Der Zugriff auf HUSAR ist ebenfalls Online möglich.

— **OMIM**-Datenbank (Online Mendelian Inheritance in Man).

Die Benutzung der GENIUS-Dienste ist für nicht kommerzielle Anwender **kostenlos** (Verbindungsgebühren werden nicht übernommen).

Wenn Sie weitere Informationen über GENIUS möchten, wenden Sie sich bitte an:

GENIUS
Molekulare Biophysik.
DKFZ
Im Neuenheimer Feld 280,
D-6900 Heidelberg.

Matthias HAGE
Tel: 06221/484-372

(W7756)G

NEUROPHILOSOPHY and ALZHEIMER'S DISEASE

La Jolla – January 11, 1991

Organised by: Patricia Churchland (UCSD) and Yves Christen (FONDATION IPSEN, Paris)

with the participation of: F Bloom (La Jolla), N Butters (San Diego), J P Changeux (Paris), P P Churchland (La Jolla), A Damasio (Iowa City), M de Leon (New York), D Dennett (Medford), M Gazzaniga (Hanover), R Guillemin (La Jolla), S Kosslyn (Cambridge), S Rapoport (Bethesda), S Savage-Rumbaugh (Atlanta), T Sejnowski (La Jolla), J L Signoret (Paris), L Squire (La Jolla), R Terry (La Jolla).

The meeting will be held at the Sheraton Grande Torrey Pines, 10950 North Torrey Pines Road, La Jolla, California, on Friday, January 11th, 1991 from 8.00 am to 5.30 pm. Registration Fee: $80.

For information or registration, contact (before December 15th, 1990): Jacqueline Mervaillie (FONDATION IPSEN), 30 Rue Cambronne, 75737 Paris Cedex 15, France. Tel: 33.1.47.34.10.95. Fax: 33.1.47.34.83.21. (W7744)C

INTERNATIONAL SYMPOSIUM

Designs for a Global Plant Species Information System

11-16th October, 1990. At E.C.C.D. Delphi Greece

The goal is international access for scientists to basic data on the taxonomy, distribution and diversity of all known plant species. Discussion starts with the needs of biological diversity, conservation, agriculture, forestry and natural product sciences. Then a range of designs will be explored for the taxonomic, botanical and computer science elements needed to put such a system in place.

PROGRAM SESSION:

1. The demand for a global plant species information system.
2. Taxonomic decision-making and data collection strategies
3. Data structures and logical designs
4. System configuration – machines and communications
5. Management, ownership and funding

Details from: G.F. Russell, Botany NHB-166, Smithsonian Institution, Washington DC 20560 USA. Tel: + 1.202-357-4362, Fax: + 1-202-786-2563
OR: F.A. Bisby, Medical & Biol. Sciences Bldg., Bassett Crescent East, Southampton SO9 3TU, UK.
Tel: + 44-703-592444, Fax: + 44-703-593939

IUBS/Linnean Society/Systematics Ass./CEC/CODATA (4689)M

ROYAL SOCIETY LECTURES

OCTOBER 1990

3 OCTOBER at 17.30: *The Philips Lecture* on **'Glass and Windows'** by Sir Alastair Pilkington, F.R.S. (also to be given at UMIST, Manchester on 25 October, the University of Liverpool on 15 November and the University of Southampton on 6 December).

11 OCTOBER at 17.30: *Evening Technology Lecture* on **'Commercial space programmes: building a sound technology base'** by Mr J.T. Rose.

18 October at 17.30 at Imperial College London: *The Leeuwenhoek Lecture* on **'How enveloped viruses enter cells'** by Dr J.J.Skehel, F.R.S.

25 OCTOBER at 17.30: *The Claude Bernard Lecture* on **'Steroid hormones and antihormones: molecular and pathophysiological aspects'** by Dr E.-E Baulieu.

30 OCTOBER at 17.30: *Lecture for the Public* on **'Black holes made simple'** by Professor M.S. Longair

Further information (tickets not required) from: The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG (Tel: 071-839 5561, ext. 278/247) (4669)M

BRITISH SOCIETY FOR PARASITOLOGY

AUTUMN SYMPOSIUM 1990

"Parasite Neurobiology"

on Friday, 28th September, 1990

at The City University, Northampton Square, London WC1Y 0HB

Convenor: Professor D W Halton (Belfast)

Speakers: Professor R J Walker (Southampton); Professor J. L. Bennett ("Norbrook Lecturer", Michigan); Dr R.J. Martin (Edinburgh); Dr I.D. Harrow (Pfizer); Professor M. O'Shea (London); Dr I. Fairweather (Belfast); Dr C. Shaw (Belfast) and Dr A.O.W. Stretton (Wisconsin).

Chairmen: Professor D.W. Lee (Leeds) and Professor J. Barrett (Aberystwyth).

Enquiries: Dr D. Rollinson, Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD.
Tel: 071-938 8887. Fax: 071-938 8754. (4674)M

THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNOUNCES

Two AACR Special Conferences in Cancer Research

DEVELOPMENTAL GENETICS OF CHILDHOOD CANCER

February 8-11, 1991
Catamaran Resort Hotel, San Diego, California

PROGRAM COMMITTEE
Chairperson: Alfred G Knudson, Jr, Philadelphia, PA
Garrett M Brodeur, St Louis, MO
Webster K Cavenee, Montreal, Canada
Stephen H Friend, Charlestown, MA
George F Vande Woude, Frederick MD

TENTATIVE SCIENTIFIC PROGRAM

KEYNOTE ADDRESS
Alfred G Knudson, Jr

REGULATION OF THE CELL CYCLE
Ira Herskowitz, San Francisco, CA
Leland H Hartwell, Seattle, WA
George F Vande Woude
Jean Y J Wang, La Jolla, CA

CONCEPTS OF DEVELOPMENT AND PATTERN FORMATION
Anthony P Mahowald, Chicago, IL
Lewis Wolpert, London, England
H Robert Horvitz, Cambridge, MA
Speaker to be announced

DEVELOPMENTAL TUMORS OF MESODERM
Webster K Cavenee
Eric Olsen, Houston, TX
David Housman, Cambridge, MA
Stephen H Friend

TUMORS OF THE NERVOUS SYSTEM
Bernd R Seizinger, Boston, MA
Garrett M Brodeur
Mark A Israel, San Francisco, CA

DEVELOPMENTAL GENETICS OF HEMATOPOIETIC MALIGNANCIES
Carlo M Croce, Philadelphia, PA
John Groffen, Los Angeles, CA
Hitoshi Sakano, Berkeley, Ca

APPLICATION DEADLINE: NOVEMBER 12, 1990

INFORMATION AND APPLICATION FORMS

AMERICAN ASSOCIATION FOR CANCER RESEARCH
Public Ledger Bldg, Suite 816
6th and Chestnut Streets
Philadelphia, PA 19106
215-440-9300
215-440-9313 (FAX)

Late applications will be accepted on a space-available basis.

MEMBRANE TRANSPORT IN MULTIDRUG RESISTANCE, DEVELOPMENT, AND DISEASE

March 10-14, 1991
Banff Centre, Banff, Alberta, Canada

CO-SPONSORED BY THE NATIONAL CANCER INSTITUTE OF CANADA

CONFERENCE CO-CHAIRPERSONS
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Victor Ling, Toronto, Canada

PROGRAM COMMITTEE
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Anna Maria Casazza, Wallingford, CT
Carol E Cass, Edmonton, Canada
Philippe Gros, Montreal, Canada
John R Riordan, Toronto, Canada

SCIENTIFIC PROGRAM

GENETICS AND DEVELOPMENT
Victor Ling, Chairperson
Louis Siminovitch, Toronto, Canada

PHOSPHORYLATION AND SIGNAL TRANSDUCTION
I Bernard Weinstein, New York, NY, Chairperson
David L Garbers, Nashville, TN
Randall R Reed, Baltimore, MD

MEMBRANE PUMPS AND CHANNELS
William T Beck, Memphis, TN, Chairperson
William A Catterall, Seattle, WA
Harvey F Lodish, Cambridge, MA
Reinhard A F Reithmeier, Toronto, Canada

MULTIDRUG RESISTANCE
Susan B Horwitz, Chairperson
Igor B Roninson, Chicago, IL
Michael M Gottesman, Bethesda, MD
Bruce A Chabner, Bethesda, MD

THE CYSTIC FIBROSIS GENE AND ITS PRODUCT
Michael J Welsh, Iowa City, IA, Chairperson
Lap-Chee Tsui, Toronto, Canada
Richard Boucher, Chapel Hill, NC
John R Riordan

P-GLYCOPROTEIN HOMOLOGUES AND DEVELOPMENT
Philippe Gros, Chairperson
Piet Borst, Amsterdam, Netherlands
Jeremy Thorner, Berkeley, CA
James M Croop, Boston, MA
Alan Cowman, Melbourne, Australia

METABOLITE AND DRUG TRANSPORT
Alan R P Paterson, Edmonton, Canada, Chairperson
Carol E Cass
I David Goldman, Richmond, VA
Stephen B Howell, La Jolla, CA
Shimon Schuldiner, Jerusalem, Israel

PERMEASE SYSTEMS IN BACTERIA AND EUKARYOTES
Giovanna F-L Ames, Chairperson
Ernest M Wright, Los Angeles, CA
H Ronald Kaback, Los Angeles, CA

A round table, "Chemomodulation of Drug Resistance," is being organized and will include Helen Chan, Toronto, Canada; William S Dalton, Tucson, AZ; and Tito Fojo, Bethesda, MD, as discussants.

APPLICATIONS DEADLINE: DECEMBER 3, 1990

(NW5812)C

RE-ADVERTISEMENT

MRC
Medical Research Council

NIMR MILL HILL LECTURE SERIES 1990-1991

All lectures will be held at **4.15 pm** in the Fletcher Hall, National Institute for Medical Research, Mill Hill, London NW7 1AA. Visitors are welcome to attend. Please contact the Director's Office (Tel: 081-959 3666) for further information and to be placed on the mailing list for these lectures.

PROGRAMME

1990

Wed. 17th October	HARTMUT MICHEL, Max Planck Institute, Frankfurt, FRG. "Photosynthetic reaction centre: structure of a membrane protein complex."
Wed. 14th November	PETER GRUSS, Max Planck Institute, Göttingen, FRG. "Mouse developmental control genes."
Wed. 19th December	**THE HARINGTON LECTURE** RICHARD FLAVELL, Yale, USA. "Molecular approaches to immune function and tolerance."

1991

Wed. 23rd January	BERT SAKMANN, Max Planck Institute, Heidelberg. "Role of receptor structure and function in plasticity of synaptic transmission."
Wed. 20th February	HUGH PELHAM, Lab. of Molecular Biology, Cambridge. "Recycling and release: protein sorting in the secretory pathway."
Wed. 13th March	DIMITRIS KIOUSSIS, NIMR, London. "Thymus targeted gene expression: a strategy for study of T-cell differentiation."
Wed. 27th March	COREY GOODMAN, University of California, Berkeley, USA. "Molecular genetics of cell adhesion and cell recognition in *Drosophila*."
Wed. 10th April	STANLEY PRUSINER, Univ. of California, San Francisco, USA. "Genetic and infectious basis of prion diseases — implications for CJD of humans, scrapie of sheep and BSE of cattle."
Wed. 15th May	KEN HOLMES, Max Planck Institute, Heidelberg, FRG. "The structure of actin."
Mon. 17th June	FRED ALT, Columbia University, New York, USA. "Recombination events during lymphocyte differentiation."
Wed. 10th July	**ROYAL SOCIETY 1991 LEEUWENHOEK LECTURE** HARRY SMITH, Professor Emeritus, Univ. of Birmingham, UK. "Microbial pathogenicity and the environment *in vivo*."

Please note that due to certain typographical errors, this advertisement is an amended version of the one which appeared in last week's issue – page 27.

(4694)M

From undergraduate to director, from immunology to oceanography, from public and private sector, nature reaches them all

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he Synthesis, Purification and Application f Oligonucleotides

Series of European Workshops

Applied Biosystems

lied Biosystems introduced its first automated DNA synthesizer in 1982. Since then technology, chemistry and instrumentation have been im- ved so that today in 1990 the DNA synthesizer user has a wide variety of choices at their fingertips. In order to discuss all aspects of DNA and A synthesis Applied Biosystems invites you to attend one of its Oligonucleotide Workshops. Each workshop will comprise of a mixture of satellite meetings and lectures. Lecture topics which will be presented are:

The history of the development of DNA synthesis chemistry.
The synthesis of DNA analogues.
The optimisation of GeneAmp™ PCR.

Throughout the day satellite sessions will explore the following topics in greater depth.

- Satellite 1 How to get the best out of your instrumentation. Topics covered will include cycle choice, design of oligos and operation of a variety of ABI DNA synthesisers including our new two and four column DNA Synthesisers.
- Satellite 2 Classical phosphoramidite chemistry including FOD™ phosphoramidites which allow base deprotection in 1 hour.
- Satellite 3 The design and synthesis of modified DNA such as anti-sense DNA and RNA and the attachment of reporter molecules for non-radioactive DNA sequencing and in-situ hybridisation.
- Satellite 4 Optimization of GeneAmp™ PCR. A representative from Perkin Elmer will be available at this workstation.
- Satellite 5 Genetic Analysis. Flourescent DNA Sequencing and Mapping and PCR fragment analysis will be discussed.
- Satellite 6 How to run a DNA synthesis Core Facility. One of our experienced core laboratory DNA synthesis customers will be available to help pass on their experiences of running a core facility.
- Satellite 7 Purification and quality control of synthetic DNA. Oligonucleotide Purification Cartridges (OPC™), HPLC, capillary electrophoresis and micro preparative electrophoresis will be available for 'hands-on' experience.
- Satellite 8 Customer Support. Our engineers will be available to discuss any aspect of the design and maintenance of our instrumentation.

There will be no charge for entrance but registration will be limited. It is recommended that you register as soon as possible by contacting one of the following people.

Date	Venue	No.Tel/Fax	Name
16th Oct.1990	London, U.K.	0925 825650/828196	Pauline Williams
17th Oct.1990	London U.K.	0925 825650/828196	Pauline Williams
19th Oct.1990	Copenhagen, Denmark	44 681800/680201	Mads Lundberg
23rd Oct.1990	Paris, France	(1) 48632444/48632282	Marie-Laure Gauchery
24th Oct.1990	Utrecht, Holland	(0) 3465-74868/74904	Jannemieke Versteegen
25th Oct.1990	Gent, Belgium	(0) 3465-74868/74904	Jannemieke Versteegen
26th Oct.1990	Munich, Germany	(0) 6151 87940/84899	Thomas Kintzinger

(4540)C

THIRD LORNE CANCER CONFERENCE

"Oncogenes and Cell Regulation"

February 14-17, 1991
Erskine House, Lorne, Victoria, Australia

Invited Speakers

Jerry Adams
Webster Cavenee
Ashley Dunn
Tom Gonda
Sung-Hou Kim
Ismail Kola
Chris Marshall
Frank McCormick
Charles Sherr

Glen Begley
Suzanne Cory
Ronald Evans
Ed Harlow
Peter Klinken
Wally Langdon
Hiroshi Maruta
Don Metcalf
Eric Stanbridge

he 3rd Annual Lorne Cancer Conference is held at the height of he Australian summer at a surf beach resort on the South Coast of Australia, 150 kms west of Melbourne. The conference will roadly cover aspects of growth regulation, including signal ransduction, proto-oncogenes and tumor suppressor genes.

Registrations

Applicants are invited to submit abstracts, some of which will be chosen for oral presentation and the remainder as posters. The deadline for applications is December 3, 1990.

For registration forms and additional information please contact

Dr. John Zalcberg
PH (613) 490 2582
FAX (613) 497 1050
Medical Oncology Department
Repatriation General Hospital
Heidelberg West, Vic. 3081, Australia

(4661)C

Monoclonal Antibodies For Clinical Use

28-29th November 1990, London

Over the past fifteen years, since their invention by Georges Kohler and Cesar Milstein, the Monoclonal Antibody has become a dominant force in molecular biology.

Over the next fifteen years, we can expect to see numerous applications in familiar roles, as well as new forms of molecules applied to new kinds of problems.

This major conference will consider the advances that can be expected in vaccines, in anti-cancer therapeutics, in drugs for treating heart attack, and in the solution of common allergies.

Chairmen and Speakers to include:

Dr R Thorpe, National Institute for Biological Standards and Control, UK
Dr K James, University of Edinburgh Medical School, UK
Dr C W Reynolds, National Cancer Institute, Frederick, USA
Dr A Gearing, British Biotechnology, UK
Dr M Baines, National Institute for Biological Standards and Control, UK
Professor C A K Borrebaeck, University of Lund, Sweden
Dr R I Lallone, Southern Biotechnology Associates Inc, USA
Professor W J Harris, Scotgen, UK
Dr S Ward, Medical Research Council, Cambridge, UK
Dr K Lambert, Celltech, UK
Dr G Chapman, Bio Products Laboratory, UK
Dr A J Macleod, Scottish National Blood Transfusion Service, UK
Dr S Raychaudhuri, IDEC Pharmaceuticals Inc, USA
Dr A Lahiri, Northwick Park Hospital, UK
Dr J Ledermann, University College London, UK
Professor H Waldmann, University of Cambridge, UK
Dr J W Kupiec Weglinski, Harvard Medical School, USA

For a full conference programme/registration details of the above events, please contact:

Renata Duke, IBC Technical Services Ltd
Bath House (3rd Floor)
56 Holborn Viaduct
London EC1A 2EX
Tel: 071-236 4080 Fax: 071-489 0849
Telex 888870 IBC G

(4695)C

NATURE

120 YEARS AGO

Experience only too clearly shows that familiarity breeds contempt. Even earthquakes are now quite appreciated in some parts of the world. Thus the *San Francisco Bulletin* of the 11th August says, "Popular prejudice is rather in favour of these lighter demonstrations of subterranean force, as they seem to stave off the heavier shocks." What next?

The Government of Honduras, in Central America, has granted to M. de la Roche for ten years the exclusive privilege of planting and exporting Corozo nut, paying two reals each hundred weight as royalty. What Corozo nut may mean, it is not easy to say.

The Smithsonian Institute of Washington has appointed a committee of scientific men to make a series of experiments to ascertain the temperature of the earth's crust at a considerable depth below the surface. For this purpose an artesian well at St. Louis is to be utilised, and as this is 3,843 feet deep some interesting results may be looked for.
From *Nature* September 22, 1870

100 YEARS AGO

One point on which nearly all witnesses seem to agree is the belief of the Tasmanians in the immortality of the soul. They evidently had not yet advanced so far as to be able to doubt it. Milligan had ascertained that the aborigines of Tasmania, previous to their intercourse with Europeans, distinctly entertained the idea of immortality, as regarded the soul or spirit of man. Robinson, who was present at the burning of a dead body, received the following explanation from a native: — "Native dead, fire; goes road England, plenty natives England." What he meant to say was that when a black fellow was dead and had been burnt, he went to England, where there are many black fellows. The name of England, *Dreany*, as a distinct country, and the home of white people, had become with them the name of a new Elysium. Others expected to reappear on an island in the Straits, and to jump up white men. They anticipated in another life the full enjoyment of what they coveted in this. Backhouse declares that they have some vague ideas of a future existence. Dove remarks that they were persuaded of their being ushered by death into another and happier state, and he considers this as almost the only remnant of a primitive religion which maintained a firm abode in their minds. However, as if to show that no account of their religious persuasions should go uncontradicted, Davies remarks, "though it is hard to believe that the natives have no idea of a future state, from every inquiry from themselves and from whites most conversant with them, I have never been able to ascertain that such a belief exists."
"The Aborigines of Tasmania", H. Ling Roth.

Excellent Copies of the **actual, natural Handwriting,** also of **Drawings, Music,** even of **elaborate Sketches, Programmes, Plans** (up to Double-Elephant size), **Shorthand,** and **Type-Writing,** are easily, quickly, and cheaply produced by the

AUTOCOPYIST

Very fluid ink, used with ordinary pen and paper. Auto-Circulars resemble *written* letters. Used at the House of Lords, &c.

AUTOCOPYIST DEPT. (A.E.T.Co., Ld.),

London Wall, London, and 52 Princess Street, Manchester.

From *Nature* September 18, 1890.

50 YEARS AGO

It has been known for many years that filtrates from moulds such species of Aspergillus or Penicillium may inhibit the growt many bacteria and in some cases show bactericidal properties. White (*Science*, **92**, 127; 1940) is now investigating the nature o bactericidal material present in *Aspergillus flavus*. Following on experiments of Prof. A. Fleming, work carried out at Oxford Chain, H. W. Florey, A. D. Gardner, N. G. Heatley, M. A. Jennings, J. Orr-Ewing and A. G. Sanders, *Lancet*, **239**, 226; 1940) has now shown that the substance penicillin which was isolated from moulds is able to exert its influence *in vivo*. Penicillin is only very slightly toxic to mice and rats. It inhibits the growth of several strains of Clostridium *in vitro*. Mice given repeated doses of penicillin were protected against lethal doses of *Streptococcus pyogenes*, *Staphylococcus aureus* or *Vibrion septique*. Penicillin is probably a new type of chemotherapeutic agent and is of interest on account of its low toxicity to animals.
"Bactericidal Action of Mould Products".

An exhibit which has proved attractive at the Centennial Exhibition of the Dominion of New Zealand is showing an aluminium plate floating in air in a strong alternative-current field and frying eggs in a pan without visible means of heating and support. An iron core is concealed beneath a table top and excited by A.C. windings so arranged and connected that they produce alternating magnetic fields, toroidal in form, of high intensity and opposite polarity. An aluminium pan floats on this field with such definition of position that considerable effort is required to move it downwards or sideways. Heat generated in the pan, by currents induced by the field, does the cooking. It is very suitable as an exhibit for publicity purposes, but at present it does not look as if it has any practical application, as a very large electrical input is required to maintain a magnetic field of suitable form and intensity.
"Electromagnetic Levitation".
From *Nature* September 21, 1940.

10 YEARS AGO

Polish scientists and university teachers have been quick to set up an independent trade union. The Gdansk accords, signed on 31 August between Vice Premier Mieczyslaw Jagielski for the Polish Government and Mr Lech Walesa for the Joint Strike Committee of the Baltic littoral, guarantees to all Polish workers the right to se up "free trade unions independent of Party and employees".

On 8 September, the new "Independent Union of Scientific, Technical and Educational Workers" (*Zwiazek Pracownikow Nauki, Technikii Oswiaty* — ZPNTO) was established, and two days later it held its first delegate meeting, with 286 delegates (one for every 50 members) representing scientific and academic institutio throughout Poland.

The establishment of the ZPNTO is far more than an act solidarity; it represents a new chance for Polish academic life regain its autonomy, and freedom of research. However, not all t Polish academic community is entirely happy about the foundatio of ZPNTO. Last week's delegate meeting was held in the buildi of NOT (*Naczelna Organizacja Techniczna* — Chief Technic Organization) in Warsaw. When, however, the delegates and othe supporters arrived (some 500 people in all), the NOT officials we unwilling to admit them. Only after lengthy negotiations were th allowed to go in, and even then only on condition that the chairma of NOT read a formal statement that NOT was acting merely proprietor of the hall and accepted no responsibility for what mi transpire.
"Polish scientists form free union".
From *Nature* September 18, 1980.

IN NEXT WEEK'S ISSUE

Solid 60-carbon molecules synthesized ■ Progress in understanding the Antarctic ozone hole ■ Phenotypic effect of the cystic fibrosis mutation ■ Solar activity in the sixteenth century ■ Calibration of a sparrow's magnetic compass ■ *In vitro* genetic amplification

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LA RECHERCHE, *versión en castellano*

Cada mes, especialistas de todos los países hacen balance de su disciplina en nuestra revista: de la Bioquímica a la Astrofísica, de la Arqueología a la Psicología, **Mundo Científico** es, por tanto, una síntesis permanente de todo lo que ocurre en todos los campos de la investigación.

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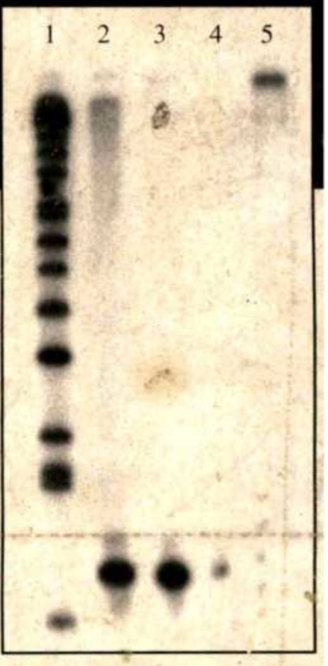

Figure Legend:
Autoradiogram demonstrating the separation of a 2.5kb amplified DNA fragment from 35mer primers using the PrimeErase Quik™ column.
Lane 1: ϕX174/Hinf I markers;
Lane 2: Amplified product before application to the column;
Lane 3: Flow-through after application of the amplified sample;
Lane 4: Flow-through after application of the washing buffer;
Lane 5: Eluted sample containing the purified 2.5kb fragment.

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DUPLICATE

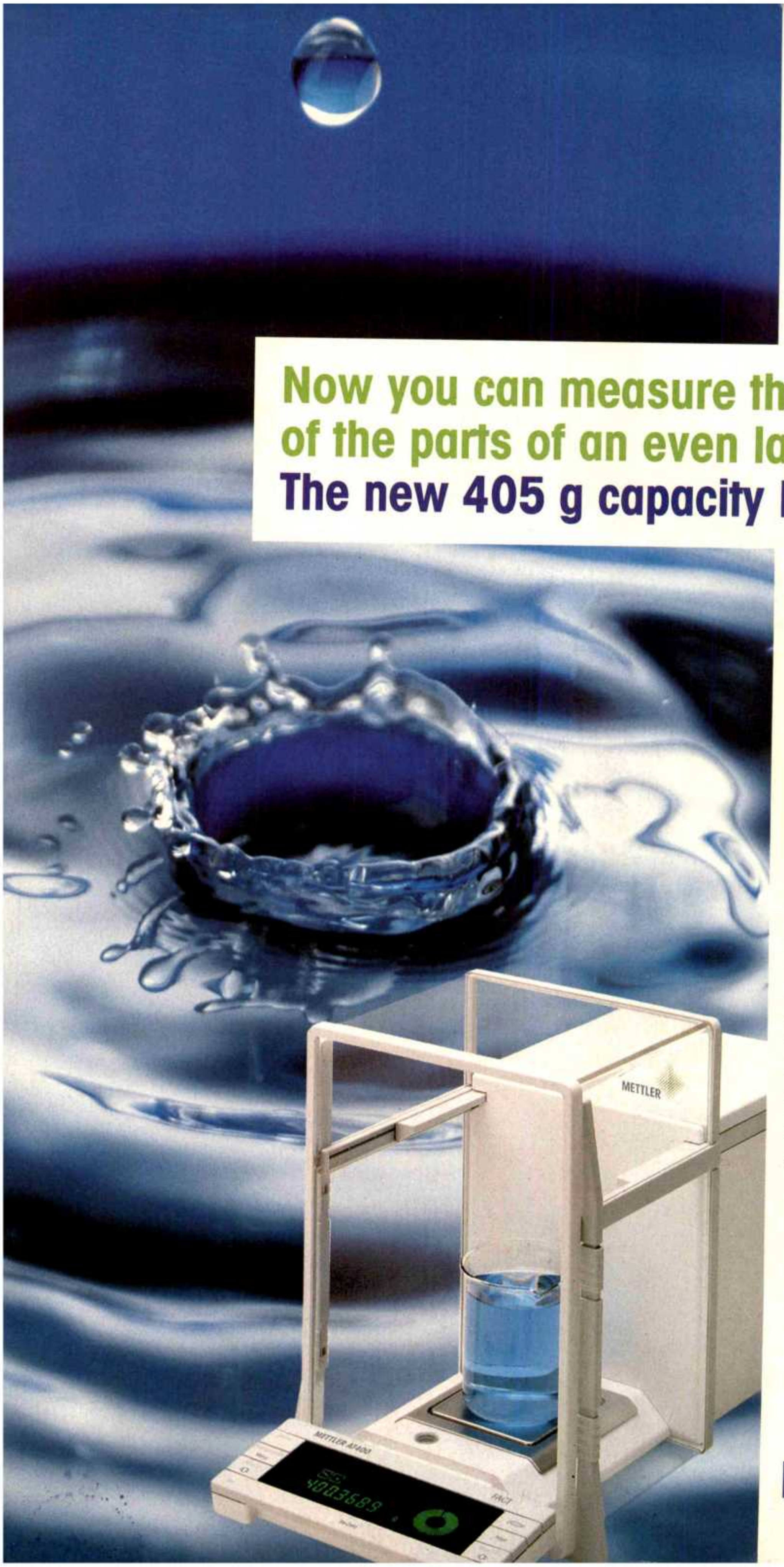
nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
Volume 347 No. 6293 11 October 1990 £2.50

THE SPECIES FLOCKS OF AFRICA'S GREAT LAKES

Light-emitting diodes from organic polymers
Mutant transcription factor Pit-1 dwarfs mice

EW JOURNALS
REVIEW



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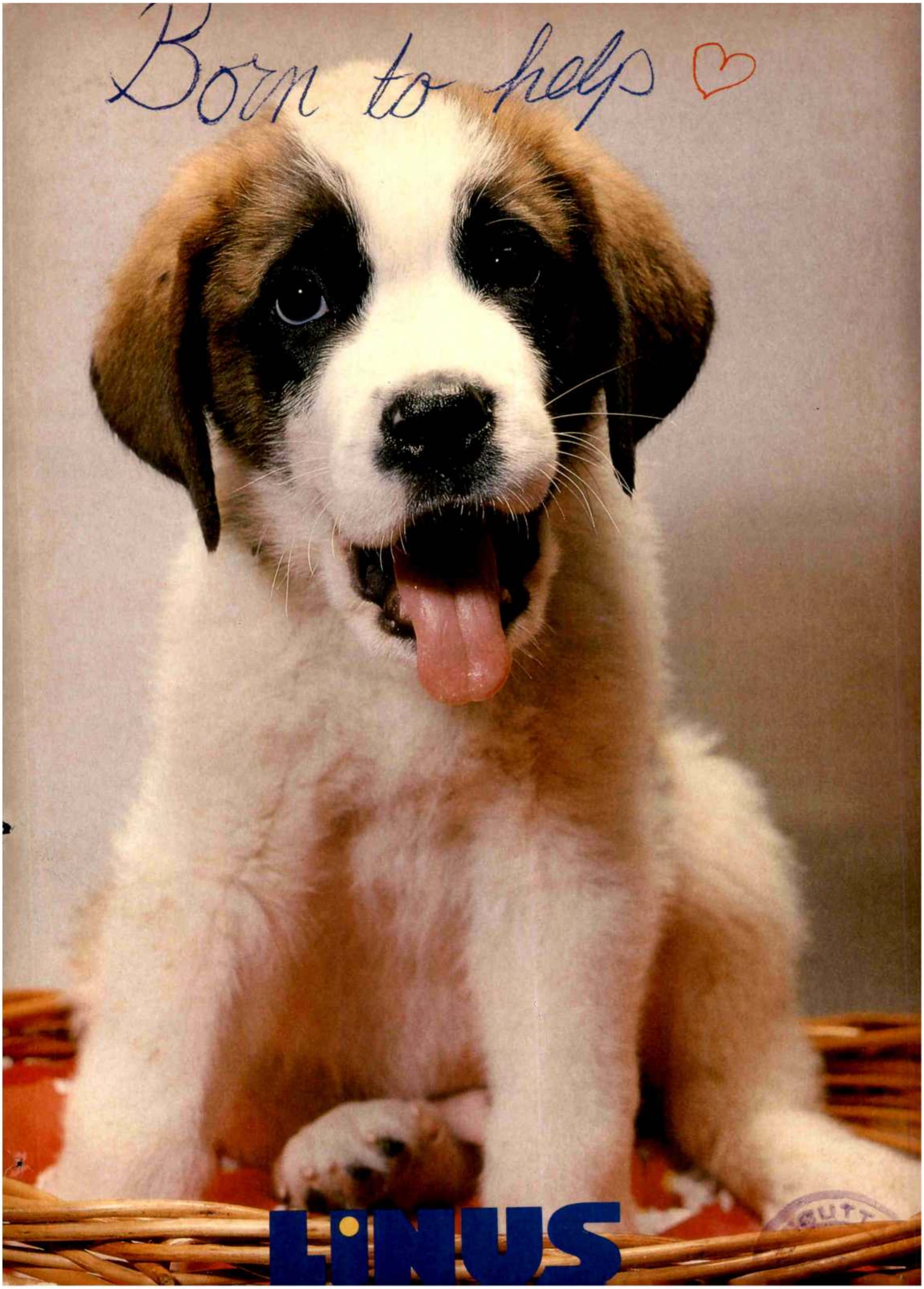

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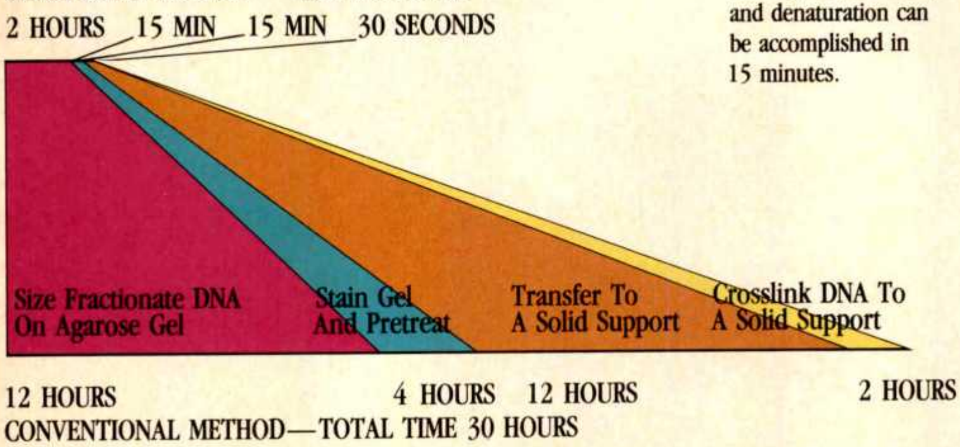

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The VAGE™ vertical agarose/acrylamide gel electrophoresis system allows the casting of agarose or acrylamide gels in the unit. Nucleic acids can be electrophoresed through a 3 mm, 0.8% vertical agarose gel in less than two hours with excellent resolution (Figure 1). Because the gels are thin, staining, depurination, and denaturation can be accomplished in 15 minutes.

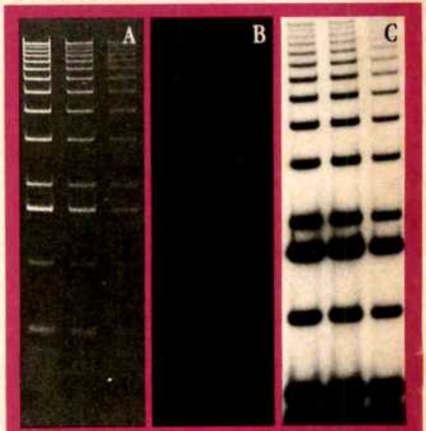


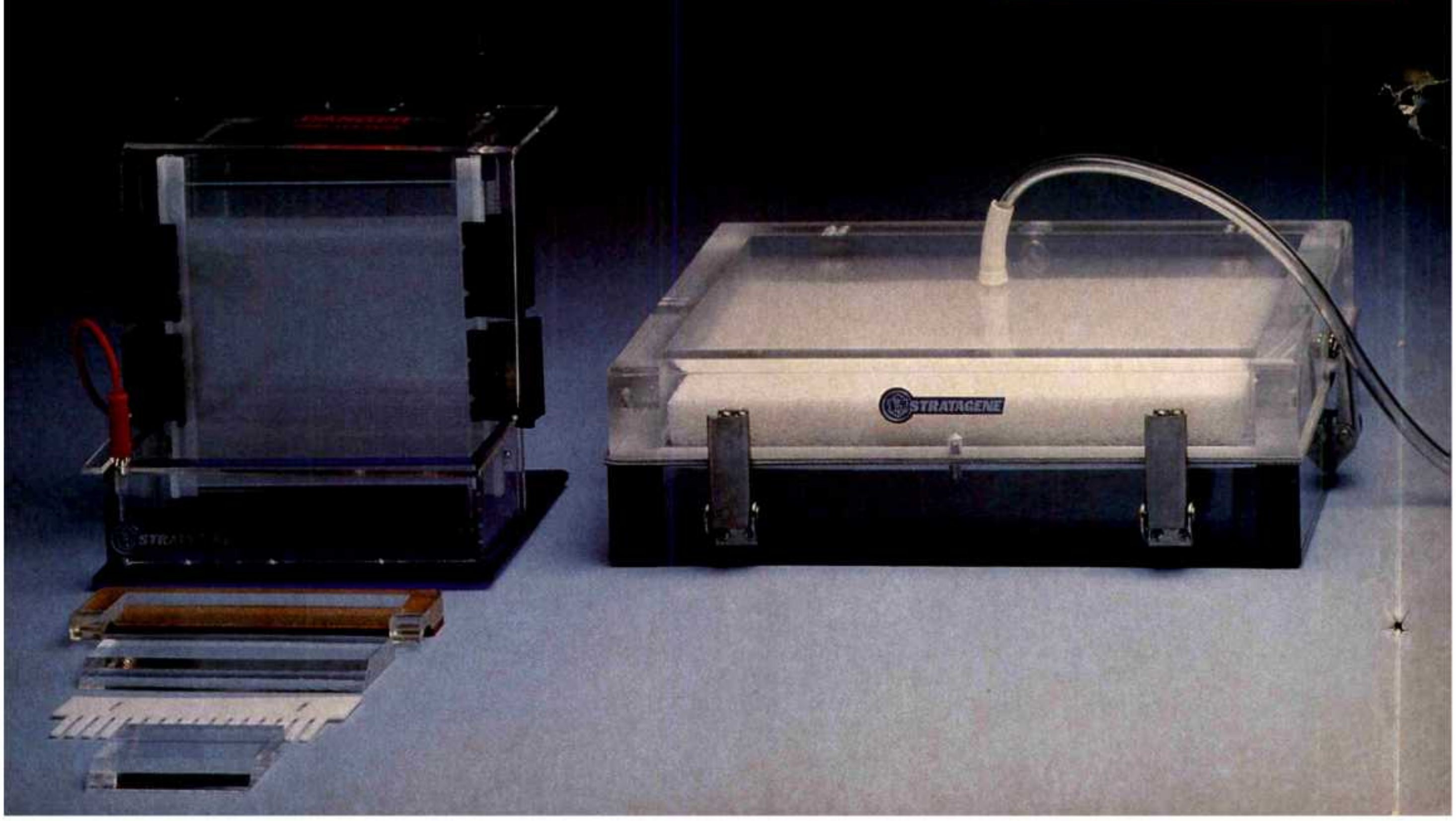
FIGURE 1:

Figure Legend: Fractionation of end labeled DNA markers on 3mm thick 0.8% agarose by the VAGE apparatus and transfer to Duralon—UV™ membranes using the PosiBlot pressure blotter.

A. Ethidium stained gel showing high resolution.

B. Same gel after pressure blotting.

C. Autoradiogram of membrane after pressure transfer.



PosiBlot™* Pressure Blotter

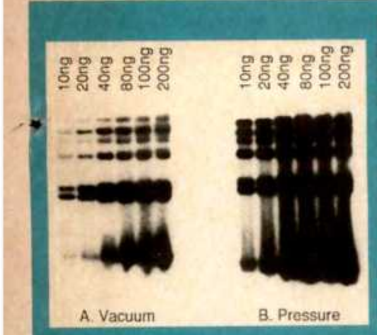


FIGURE 2:

Figure Lengend: ^{32}P end-labeled lambda Hind III markers were electrophoresed in 0.8% agarose. The DNA was then transferred to a nylon membrane with a vacuum blotter at 30mm Hg below atmospheric or with the PosiBlot pressure blotter at 100mm Hg above atmospheric. Both transfers were carried out for 15 minutes. As can be seen, pressure blotting transferred significantly more DNA in the same period of time, especially in the higher molecular weight range (largest band is 23 kilobases).

The PosiBlot™ positive pressure blotter permits the transfer of nucleic acids in 1/3 the time of vacuum blotters and 1/50 the time of capillary blotting (Figure 2). Pressure blotting does not dehydrate gels as do other methods. This allows the use of substantially higher pressure differentials, compared with vacuum blotting, without gel collapse. The PosiBlot apparatus reduces blotting time to 15 minutes.

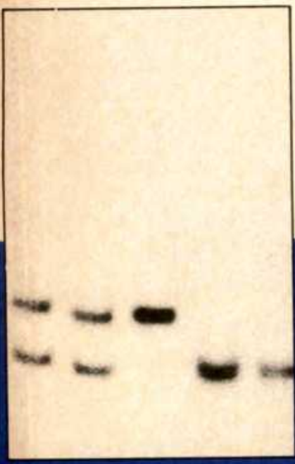


FIGURE 3:

Figure Legend: Autoradiogram showing the resolution of 2.8 and 1.3 Kb Msp I RFLP alleles revealed by a cystic fibrosis human DNA probe using the VAGE, PosiBlot and Stratalinker all in 2.5 hours.

Stratalinker™* UV Crosslinker

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DUPLICATE

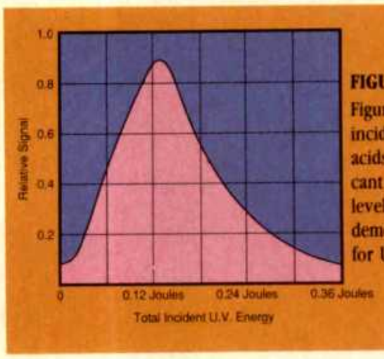

FIGURE 4:

Figure Legend: The effects of altering the incident energy for crosslinking nucleic acids to nylon membranes. The significant drop in signal intensity at energy levels below and above 0.12 Joules demonstrates the limited optimal range for UV treatment.

Stratagene offers a full selection of nitrocellulose, reinforced nitrocellulose and nylon membranes. Each membrane is stringently lot tested to ensure consistency when performing Northern and Southern blotting. Please call Technical Services for detailed information on Stratagene's time saving blotting systems and membranes.

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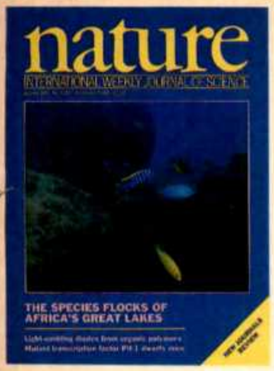
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INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
THE SPECIES FLOCKS OF AFRICA'S GREAT LAKES

nature

11 October 1990
Vol. 347 Issue no. 6293

◀ A sample of the variety of fish in Lake Malawi. On page 550 Meyer *et al.* report on mitochondrial DNA variation in the cichlid fish of the great lakes of East Africa. These 'species flocks' are regarded as natural laboratories for the study of speciation, and the new data may resolve important questions relating to their origins. See also News and Views. Photo: Andreas Spreinat

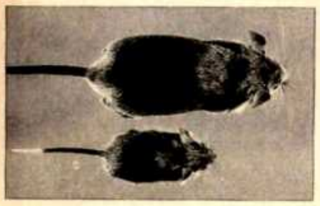
THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

Flat-screen polymer?

The development of an electrically pumped luminescent diode utilizing a conjugated polymer (pages 539 and 518) is a step towards realizing the potential of organic semiconductors. The new polymer has structural and optoelectronic properties suited to large-area light-emitting displays.

Organic growth

Two dwarf mutant mice are shown to have mutations in the *pit-1* transcription factor gene, which encodes a protein containing a POU homeodomain and is essential for pituitary differentiation. This demonstrates a direct link between a transcription factor and commitment and progression events in mammalian organogenesis. Page 528.



Paper tiger

In the battle between printed journals and electronic information systems, the casualties so far have been on the electronic side. Leading off this year's New Journals Review (on traditional paper), Michael Buckingham asks why. Page 581.

CFC replacements

A simplified laboratory method for determining the ozone depletion potentials of halocarbon compounds is described on page 541, and used to estimate the lifetimes of two halocarbons suitable for use in fire extinguishers and one intended as solvent. All three new compounds are broken down efficiently in the troposphere, and so show promise as replacements for today's CFCs and halons.

Island race

Long-distance dispersal of seeds by birds may be responsible for the great distance between two subspecies of the flowering plant *Peperomia berteroana* found respectively in the Juan Fernandez Islands in the Pacific and 5,000 km away on the Tristan da Cunha archipeligo in the souh Atlantic. Page 549.

Escape clause

Variants of foot-and-mouth disease virus selected for their ability to escape neutralization by a monoclonal antibody are shown to adopt a novel mechanism of antigenic variation via sequence changes at residues not directly involved in antibody binding. The antibody binds to a distinct site, which crystallography shows adopts a different conformation in the variants from the wild-type virus. Page 568.

We have a problem

The so-called solar neutrino problem, the fact that detection rates for solar neutrinos are about one-third of those predicted by standard models, is tackled by Elsworth *et al.* using precise seismological measurements of the Sun's core. They conclude that the 'problem' must be resolved within neutrino physics, not by revising current models of the Sun's structure. Page 536.

Sun and sea

Ocean transparency measurements from the Nimbus-7 satellite, together with climatological density and heat-flux data, show that solar radiation in visible frequencies, usually assumed to be absorbed at the sea surface, in fact penetrates tens of metres beneath the surface. This may account for the discrepancy between predicted and observed sea surface temperatures. Pages 543 and 515.

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Britain gallops out as America reins in 501

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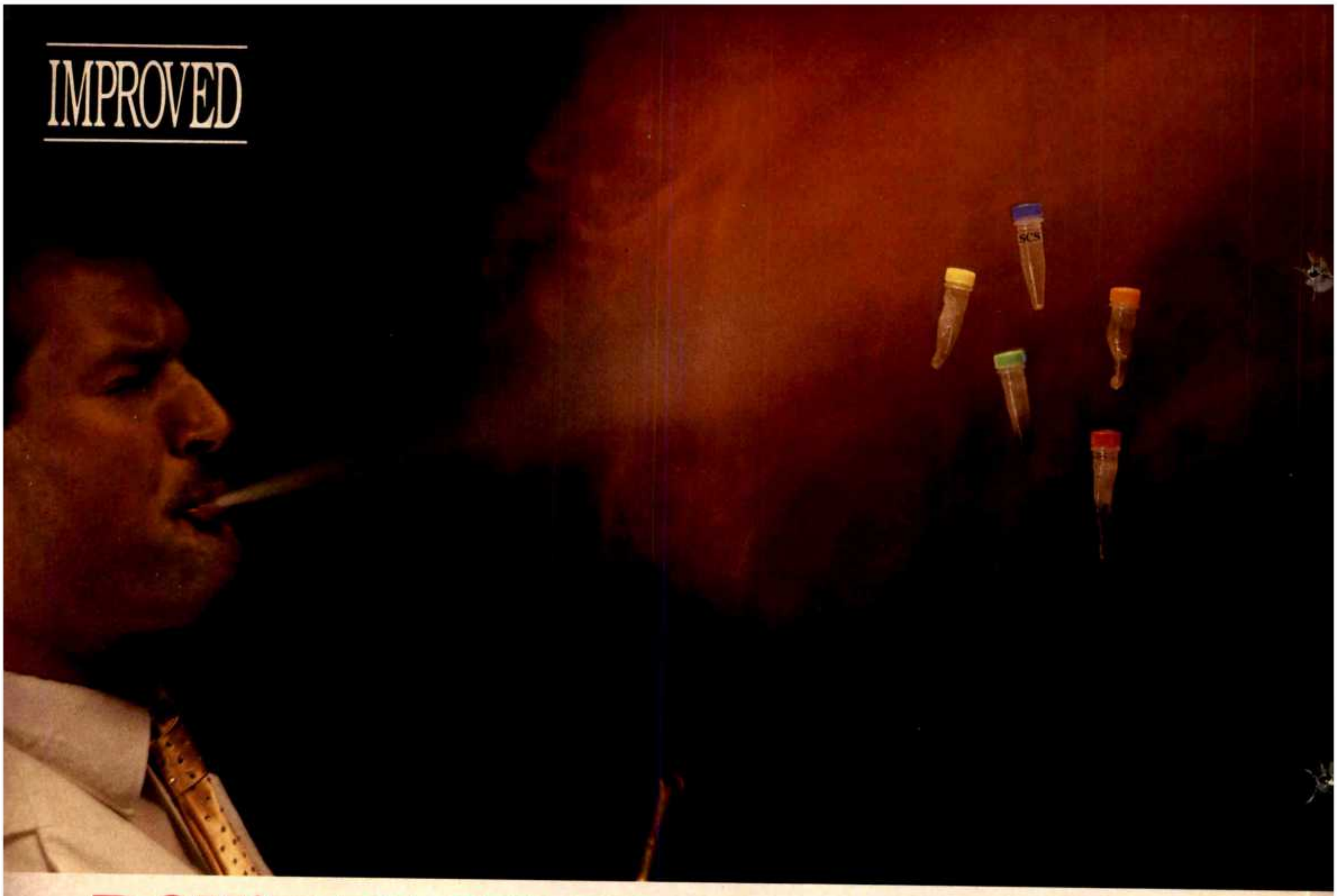

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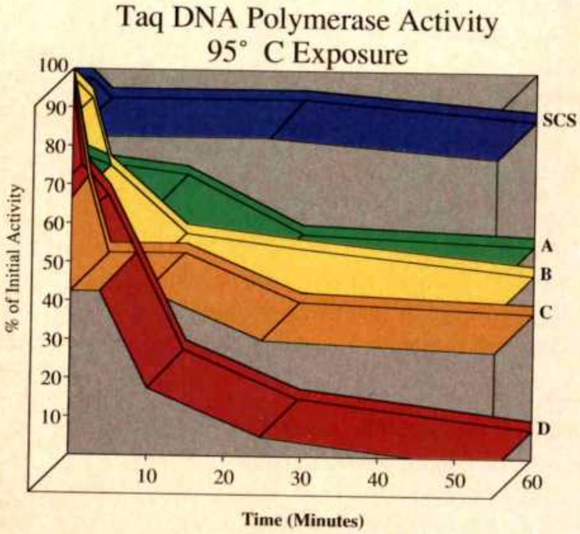

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F Michel, A D Ellington, S Couture & J W Szostak **578**

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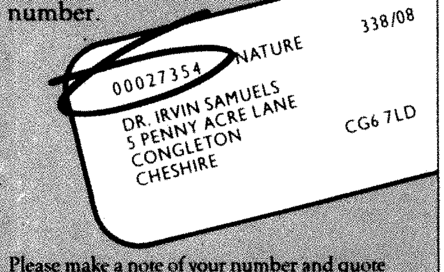

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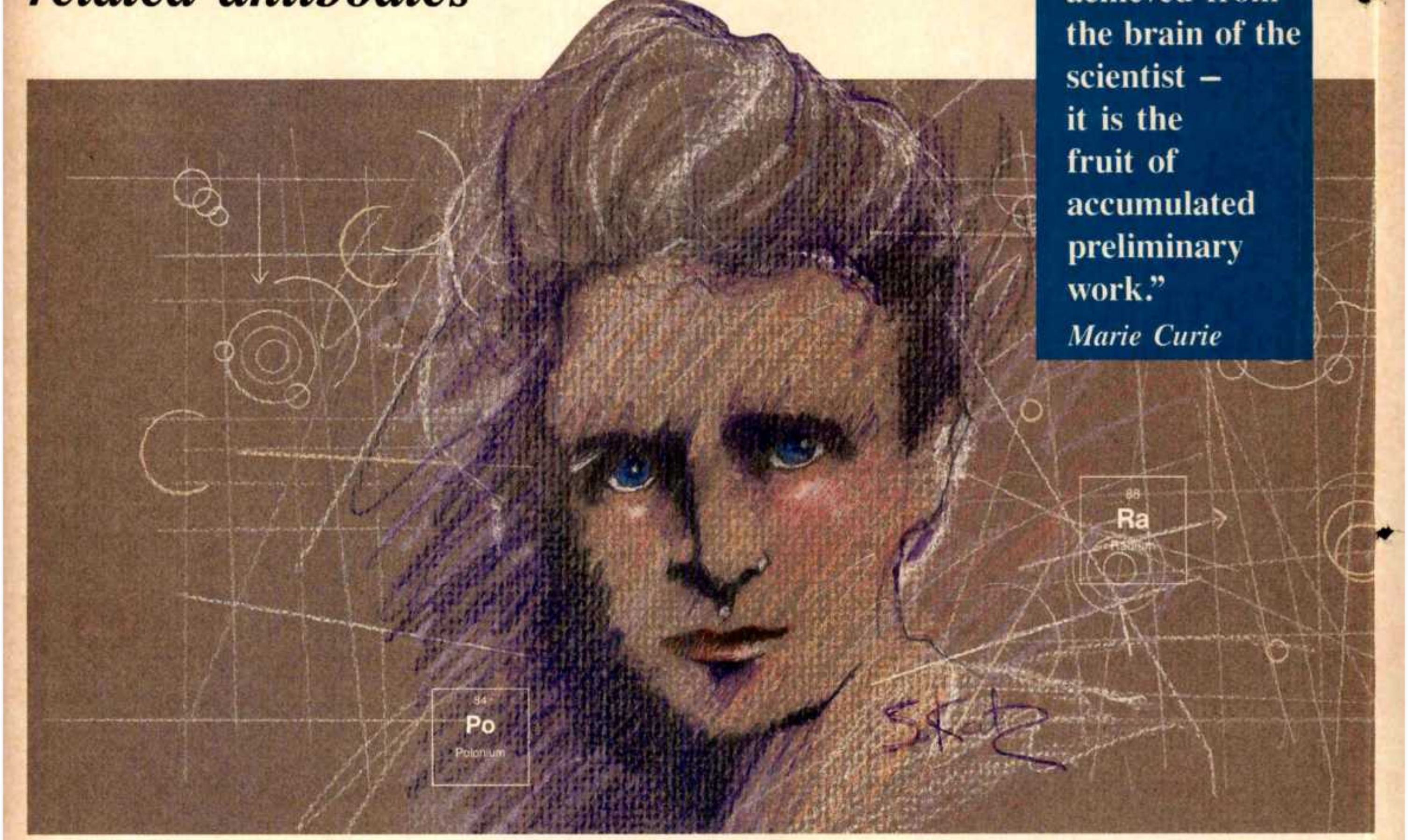

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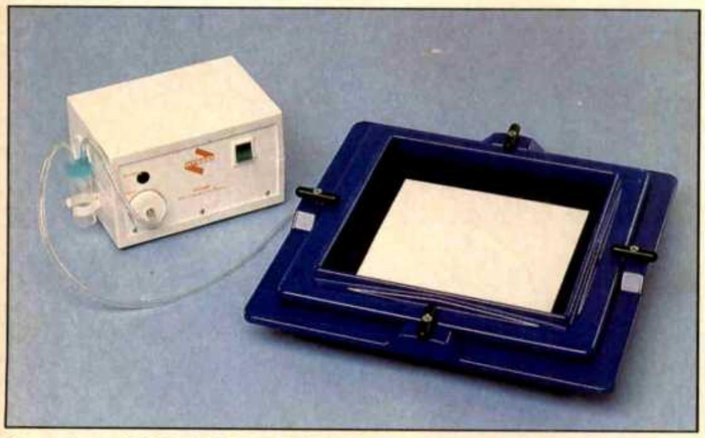

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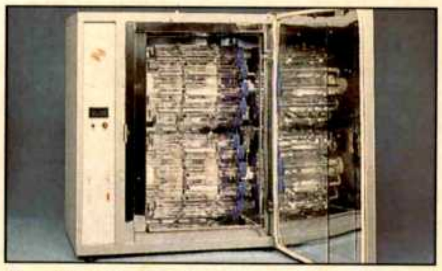

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BLOT PROCESSING

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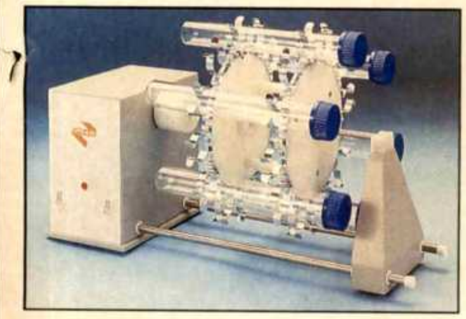

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BLOT PROCESSING

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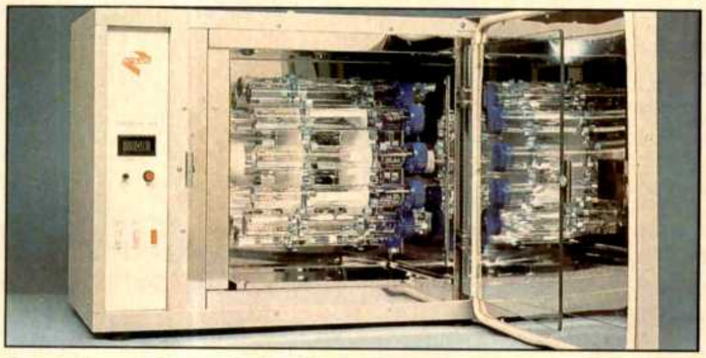

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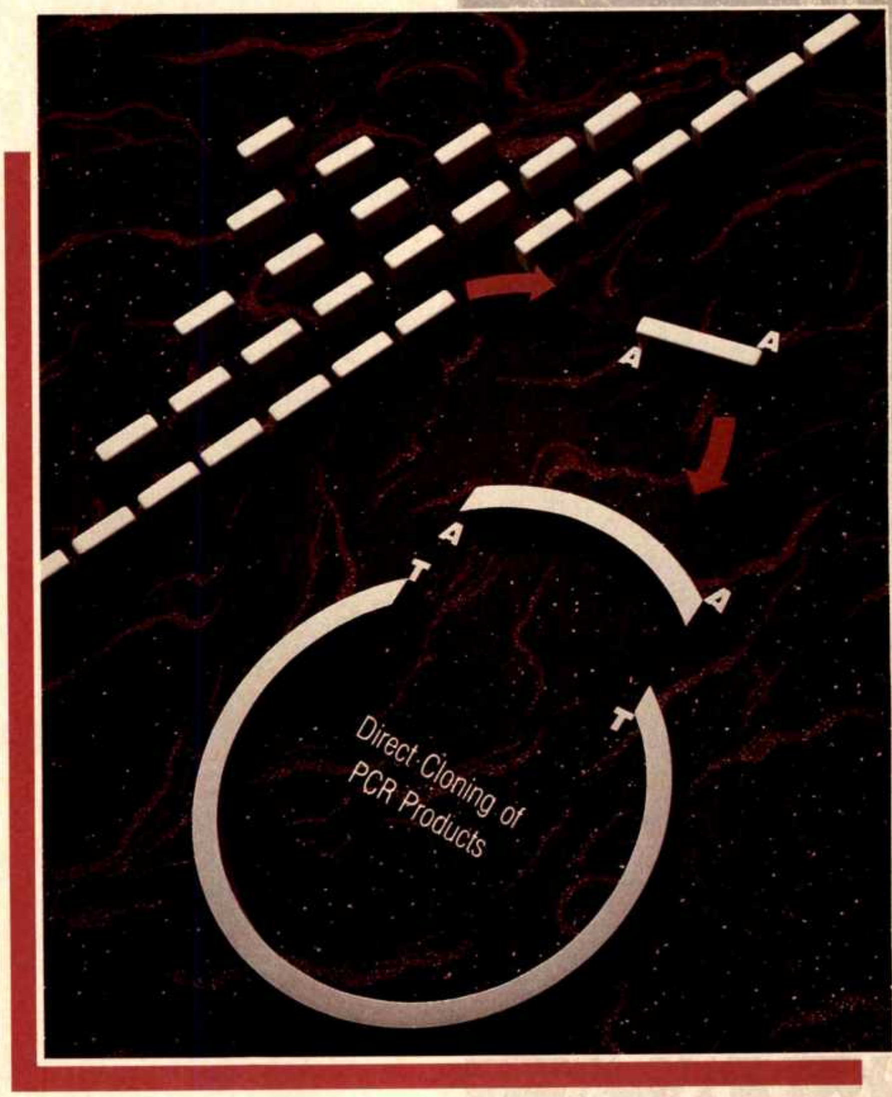
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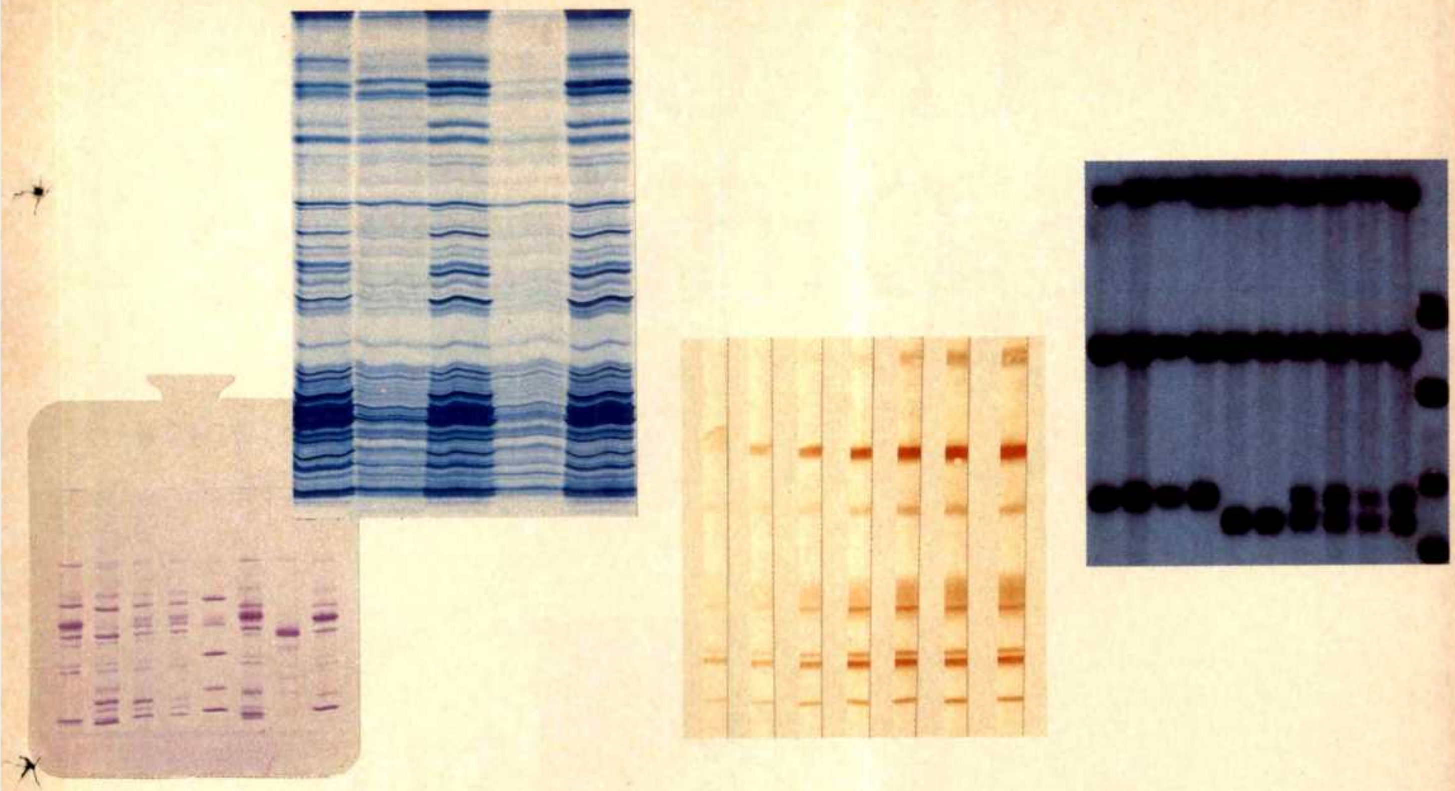

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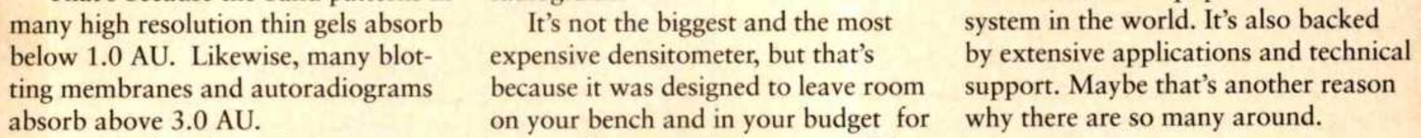

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Children's Hospital of Michigan
3901 Beaubien Boulevard
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(313) 745-5570

Citing Contract #N01–CP–85645

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Contact: Ms. Elizabeth Donley
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5221 River Road
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(301) 657-8169

Citing Contract #N01–CP–61020

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Contact: Coordinator for Research Resourc
Biological Carcinogenesis Branch,
DCE, NCI, NIH
Executive Plaza North, Room 540
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Contact: Registry of Experimental Cancers
National Cancer Institute
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Contact: Harold E. Seifried, Ph.D.
Chemical and Physical
Carcinogenesis Branch
DCE, NCI
Executive Plaza North, Room 700
Bethesda, MD 20892
(301) 496-5471

Cost: Inquire

■ Chemical Carcinogen Reference Standard Repository: Reference quantities of over 750 compounds are available. The newest additions are dilute aqueous standards of PAH deoxyguanosine-3′-monophosphates for Randerath ^{32}P post labelling assays. Other classes of available compounds are: fecapentaenes, food mutagens, polynuclear aromatic hydrocarbons (PAH), PAH metabolites, radiolabeled PAH metabolites, nitrogen heterocycles, nitrosamines/nitrosamides, aromatic amines, aromatic amine metabolites, azo/azoxy aromatics, inorganics, nitroaromatics, pesticides, pharmaceuticals, natural products, dyes, dioxins and chlorinated aliphatics. Data sheets provided with the compounds include chemical and physical properties, analytical data, hazards, storage, and handling information. Catalog available upon request.

Contact: Manager, NCI Chemical
Carcinogen Repository
Midwest Research Institute
425 Volker Boulevard
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stein-Barr virus (EBV) in Burkitt's
nphoma, nasopharyngeal carcinoma, and
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e TVER is able to adjust its collection to
ilitate the development of new collaborative
dies. In addition, some samples are available
reagents and independent research. The
ost extensive collections are serum samples
m patients with Burkitt's lymphoma (sera
m more than 1,000 patients).

ntact: Dr. Paul H. Levine
Environmental Epidemiology
Branch, DCE, NCI, NIH
Executive Plaza North, Room 434
Bethesda, MD 20892
(301) 496-8115

st: Free to Collaborating Investigators;
Others: Dependent on Processing
Time

The National Cancer Institute has available
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leges of Veterinary Medicine in North
erica (also known as the Veterinary
dical Data Program). This unique registry
eterinary medical information represents
ent data on animals seen at collaborating
erinary teaching facilities; 3 million hospital
sodes have been abstracted and com-
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ease information is coded using the scheme
ne Standard Nomenclature of Veterinary
ease and Operations. The computer tapes
be made available upon request.

ntact: Dr. Howard M. Hayes
Environmental Epidemiology
Branch
Epidemiology and Biostatistics
Program
Division of Cancer Etiology
Executive Plaza North, Room 443
Bethesda, MD 20892
(301) 496-1691

st: Inquire

■ The National Institute of Allergy and Infectious Diseases and the National Cancer Institute have developed a repository of biological specimens from homosexual men. The specimens were collected through contracts with five major U.S. universities for studies of the natural history of acquired immune deficiency syndrome (AIDS).

Information about applying for collaborative use of these specimens is available from the NIAID Project Officer or the NCI Co-Project Officer.

Contact: Chief, Epidemiology Branch,
AIDS Program
National Institute of Allergy and
Infectious Diseases
CDC Bldg., Room 240
National Institutes of Health
Bethesda, MD 20892

or to Chief
Extramural Programs Branch, EBP,
Division of Cancer Etiology, NCI
Executive Plaza North, Room 535
Bethesda, MD 20892

■ The Epidemiology and Biostatistics Program of the National Cancer Institute has developed the Observed versus Expected (O/E) software system which calculates: (1) the number of observed events (e.g. cancer cases or deaths) in a study group at risk; (2) the number of expected events in a study group based on the rate of occurrence in some standard or referent population; (3) the ratio of observed to expected events; and (4) the significance of this ratio. The system is user friendly and capable of executing a series of calculations by different variables such as age, time group, date of exposure, age at date of exposure, duration of exposure, year relative to entry and cause of event. The O/E System provides tables by race, sex and user defined variables, allows user defined latency intervals and accepts standard or user prepared rates. O/E is written in COBOL and is exportable to most mainframes.

Contact: Ruth Wolfson
Epidemiology and Biostatistics
Program
Division of Cancer Etiology, NCI
Executive Plaza North, Room 531
Bethesda, MD 20892
(301) 496-1606

Cost: Free to investigators interested in epidemiologic research.

■ Human fibroblast cultures from individuals at high risk of cancer, members of cancer-prone families, and normal family members are available. Collection is historical with unknown viability. Catalogue unavailable. Information requests should include potential use of cultures.

Contact: Chief, Family Studies Section,
EEB, DCE, NCI, NIH
Executive Plaza North, Room 439
Bethesda, MD 20892
(301) 496-4375

Cost: Free to collaborating investigators
Others: $70/cell line.

nvironmental Cancer

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rmation System (CCRIS) is available
ne through the National Library of
licine's Toxicology Data Network
XNET) system. Through an interagency
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ved from published review articles, on-going
ent awareness survey of primary literature,
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es, the IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, and special studies and reports.

Contact: Dr. Thomas P. Cameron
Office of the Director
Division of Cancer Etiology
National Cancer Institute
Executive Plaza North, Room 712
Bethesda, MD 20892
(301) 496-1625

Cost: Inquire

■ The Special Assistant for Environmental Cancer, Office of the Director, announces the availability of a limited number of copies of the following publications, which have been prepared under contract to NCI:

Survey of Compounds Which Have Been Tested for Carcinogen Activity, PHS-149, 1987-1988

And Proceedings of the Fourth NCI/EPA/NIOSH Collaborative Workshop: Progress on Joint Environmental and Occupational Cancer Studies, 1986

Contact: Ms. I.C. Blackwood
Office of the Director
Division of Cancer Etiology
National Cancer Institute
Executive Plaza North, Room 712
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Cost: Free to investigators interested in environmental cancer

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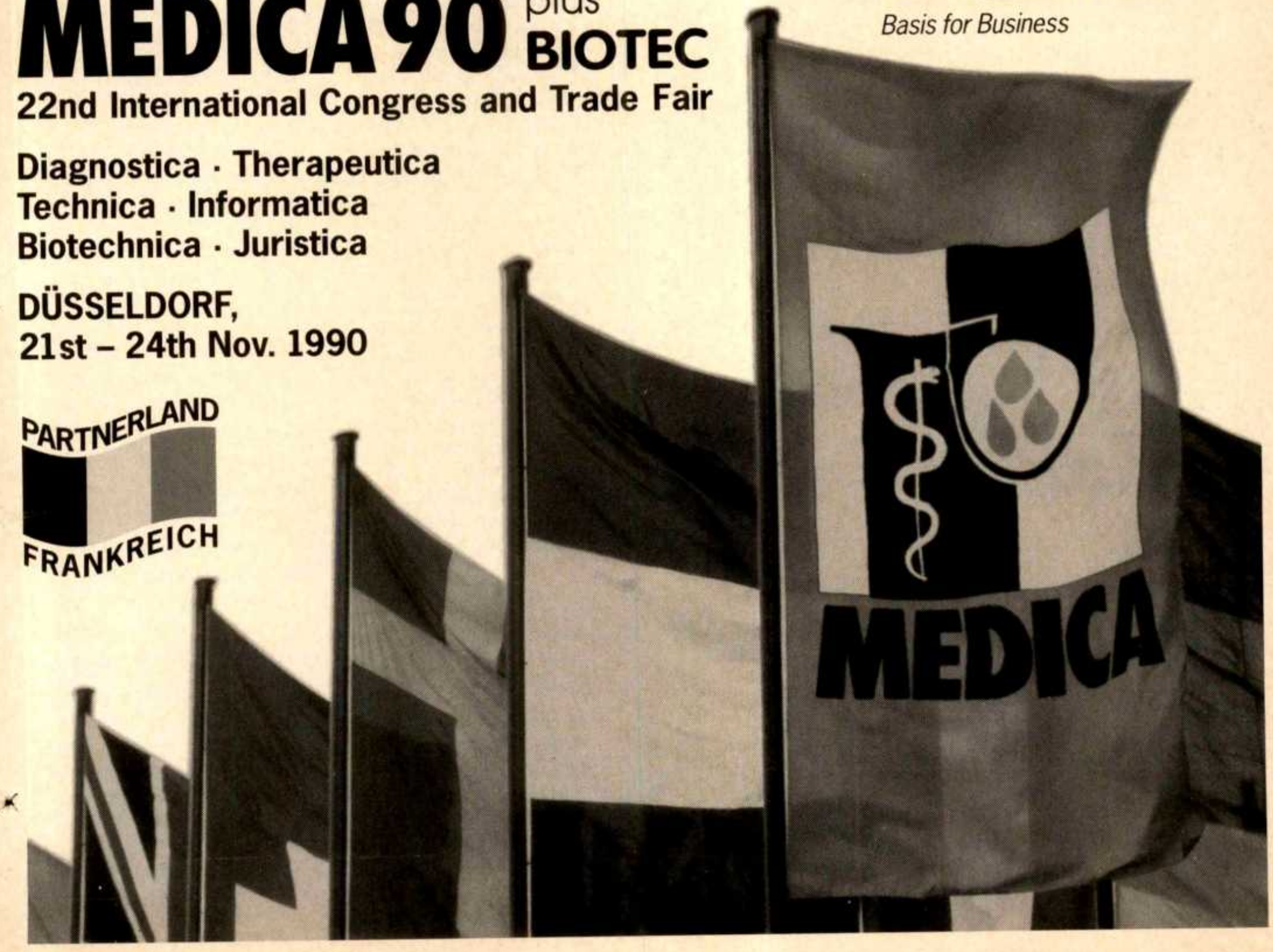

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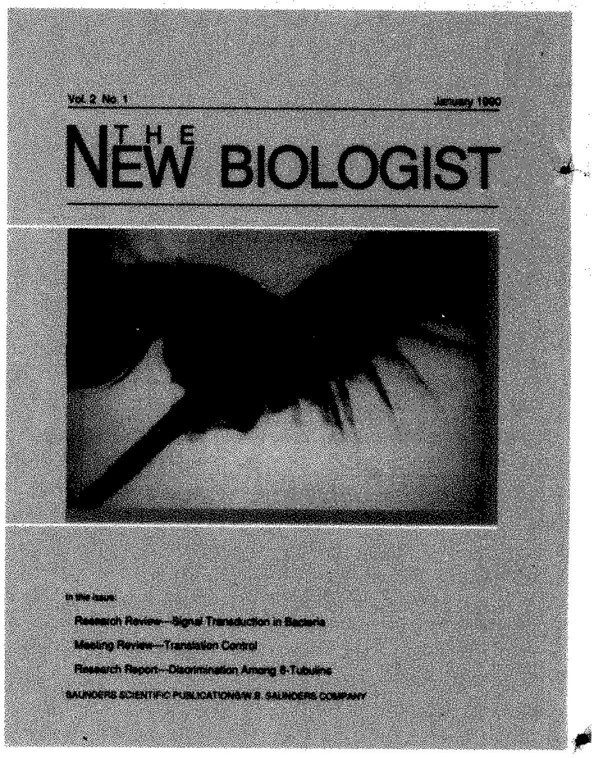
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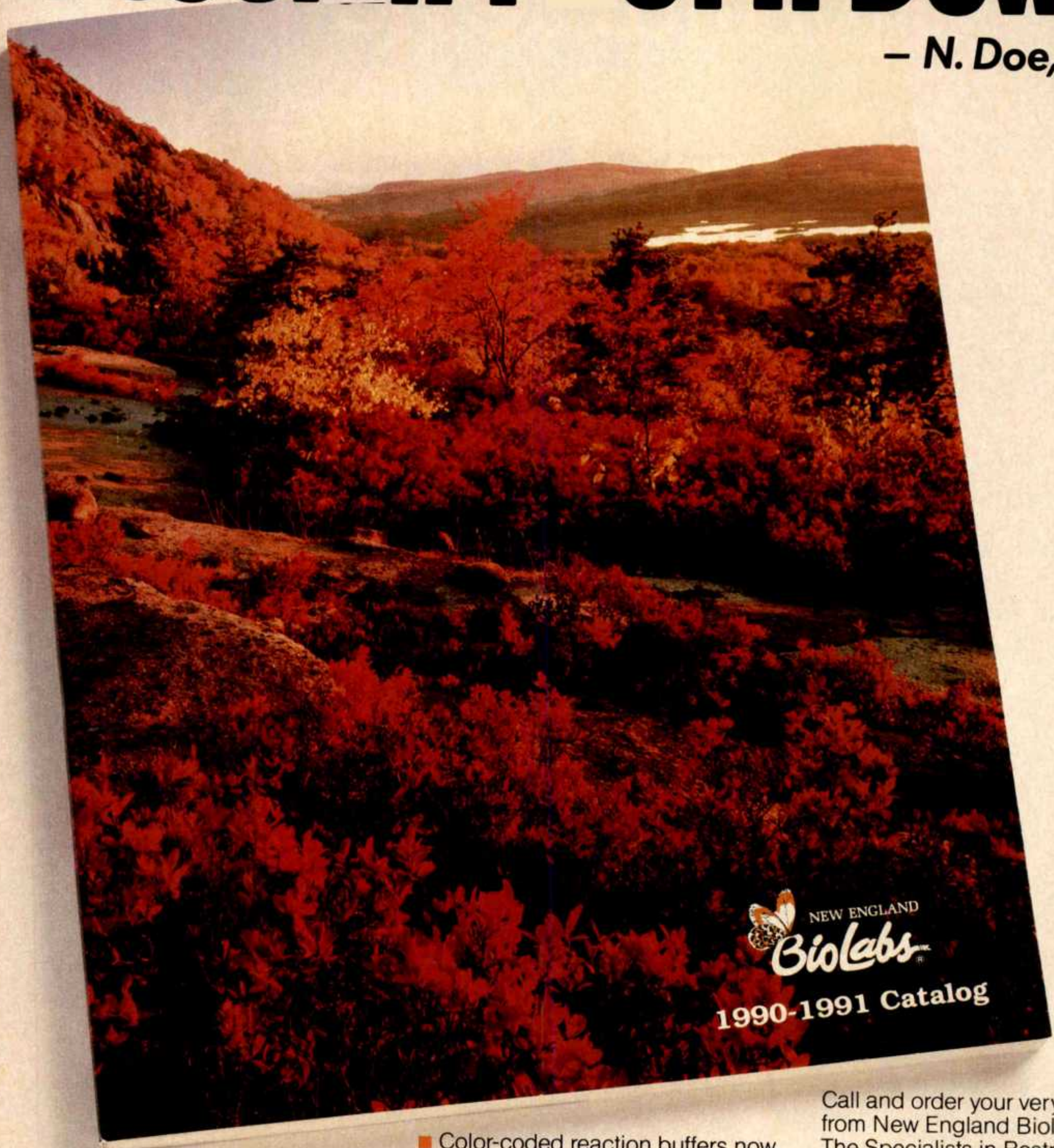

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A tale of two budget systems

The United States may have something to learn from Britain's different handling of the economy, but the effects of Britain's entry into the European Monetary System will not be known for some time.

THE founding fathers of the United States, with the Boston Tea Party's slogan ringing in their ears, cannot have imagined a time when there would be no taxation even with representation. Yet that is what last weekend's farce in Washington shows now to be the case. When the House of Representatives last Thursday threw out the budget bitterly negotiated between the White House and the Congress over four painful months, its members were in effect complaining that it is more than can be expected of flesh and blood to require the people's representatives to face an election (a month from now) when they have recently given assent to measures that may be unpopular with many voters. No crisis, they were saying, can be worse than not being re-elected.

There may have been some regret in the United States, at the White House in particular, at last weekend's demonstration that in Britain, which provoked the Boston business, matters are still ordered differently. With no specific word of warning, the Chancellor of the Exchequer, Mr John Major, declared that Britain would promptly become an effective member of the European Monetary System — probably the most significant change in Britain's monetary constitution since the abandonment of the Gold Standard in 1931. Again there are electoral calculations, but in a system in which governments themselves decide when to stand for re-election, only the unfolding of events will show what these may have been.

The US budget dispute is the more serious because the US economy is more influential than the British, because the direct effects of a failure to resolve it will be directly harmful, not least to the welfare of the scientific enterprise in the United States — and because there is also a danger that a solution, if unintelligently put into effect, may itself have consequences that are calamitous.

The outline of the crisis is well known — the federal government outspends its revenue by between $150,000 million and twice as much, depending on how (and by whom) the arithmetic is done. For the past half decade, the Congress has bound itself either to effect regular reductions of the deficit or to have government spending cut indiscriminately across the board. After promising during the 1989 election that taxes would never be increased, and having eaten humble pie, President George Bush is now rightly demanding that the Congress should give him a budget, not sequestration by default.

On that the president has no choice. The deficits of the Reagan years were a device for making people in the United States feel good, able to enjoy public services they had not paid for. The sleight of hand depended on the willingness of people elsewhere either to lend directly to the US government or to buy tangible assets in the United States, releasing dollars for the US Treasury to borrow. But that source of funds has now dried up.

The whole world is short of money, and US dollars are cheaper than they have been for years. Yet the United States is heading for recession, when a federal deficit would make keynesian sense, as would the further reduction of interest rates to which the Federal Reserve is edging. Has Bush's devotion to a budget settlement, which would have been proper in any of the ten years past and perhaps even when this year's budget cycle began in February, been overtaken by events? The United States would find a serious recession uncomfortable, but many among the rest of us would find it calamitous as well.

At least in some procedural respects, the British government's problem has something in common with that in Washington. As in the United States, economic activity is rapidly decelerating, but the government is having to squeeze excess credit out of the system by means of high interest rates, upwards of 15 per cent until last Friday. They could not have been allowed to rise much higher without exciting the wrath of voters, and might have been economically dangerous as well. So why not join the Exchange Rate Mechanism, the system by which the European Monetary System ensures that the currencies of the European Communities' members remain more or less fixed in relation to each other?

The decision to do just this, pulled like a rabbit from a hat on the eve of the government's annual meeting with its party supporters, has already been used to bring down interest rates by 1 per cent. That benefit, probably the first of several of the kind, is immediate. The price that will have to be paid — the painful business of keeping inflation within reasonable bounds — will on the other hand be delayed by several months. There are piquant side-issues, not least the prime minister's much advertized coolness to this scheme and the possibility that, in the changed circumstances, Major may be better able to make his sensible version of the case for a single European currency. Nevertheless, Bush is fighting to resist externally imposed sequestration just when Major is tying Britain to an external source of discipline. □

NIH launches surprise Gallo investigation

- Existence of independent viruses accepted
- Missing data and misleading statements

London & Washington

IN A surprise announcement, the National Institutes of Health (NIH) last week said that it had decided to convert its inquiry into the conduct of National Cancer Institute researcher Robert Gallo into a "formal investigation", even though it had resolved or "shown to be without substance" several of the "publicized issues and allegations" concerning Gallo's role in the discovery of the AIDS virus.

According to a confidential letter sent by NIH acting director William Raub to Gallo, findings to date indicate that there is "substantial reason to believe that scientific misconduct may have occurred in some instances" during the fiercely competitive hunt for the AIDS virus in 1983 and 1984.

But the specifics of the coming investigation, given in the confidential letter to Gallo, appear to indicate that any "misconduct" involves issues of misleading wording and missing data, rather than the broad issue that Gallo 'stole' the virus from competing researcher Luc Montagnier of the Pasteur Institute in Paris.

Vanishing motive

According to Gallo, the issue has now "been conclusively taken care of" because the inquiry accepts that his laboratory had isolates of AIDS virus from sources other than the Pasteur Institute at the time the virus was being identified as the cause of AIDS. Raub's statement says that "the inquiry team has concluded that Dr Gallo had a substantial number or HIV detections and isolations from several different sources at the critical time . . .". With viruses from other sources growing in his laboratory, Gallo claims that he would have no reason to steal the French virus, known as LAV.

Gallo is, however, willing to accept that the extreme similarity of LAV and the virus he isolated — named HTLV-IIIB — could indicate that LAV entered his own culture through accidental contamination. But viral contamination is a common problem and not, says Gallo, "a question of ethics".

The NIH investigation will attempt to resolve the issue of where Gallo's virus came from. According to Raub's prepared statement, the investigation is to "include testing of a number of biological samples in an effort to determine the origins of HTLV-IIIB, the virus that Dr Gallo and his colleagues used to develop the [AIDS] blood test." Gallo says that the testing process will attempt to determine whether any of the 10 viruses known to have been independently isolated in his laboratory at the time could have provided the source of HTLV IIIB. The investigation will also inlcude Gallo's chief virologist, Mikulas Popovic, who had been responsible for growing viruses during the critical 1983–84 period.

Gallo's lawyer, Joseph Onek, says that the fact that NIH officials are addressing the issue at all proves that they consider it an open question. "When you're saying that you're looking for the source of HTLV-IIIB that means you are not necessarily accepting contamination", but are considering the possibility of independent isolations, he says.

According to Gallo, it may be difficult for NIH to come up with a final judgement on the origin of his virus. Although only 10 separate viruses were known to have been combined in the viral "pool" maintained by Popovic from which HTLV-IIIB was derived, some 78 other viral isolates were known to be in the laboratory at the time — and contamination from them is as likely as from LAV, says Gallo. Also, two of the original 10 isolates that went into the pool are no longer in existence, so their similarity to HTLV-IIIB/LAV cannot be checked.

The issue of missing data to be raised in the formal investigation focuses on a paper by Popovic and Gallo published in May 1984. The paper was the first of a series of four key reports published by Gallo's laboratory in a single issue of Science; taken together, they served to establish HTLV-IIIB as the cause of AIDS.

John Crewdson, the investigative reporter whose 16-page article in the Chicago Tribune newspaper stimulated the NIH inquiry, alleges that there are important discrepancies between the information published in the paper and Popovic's laboratory notes. In particular, he maintains that a table describing the properties of 5 viral isolates, growing in Gallo's laboratory before publication is misleading.

The questions about the paper bear strongly on the precise status of various viral isolates that were being kept in Gallo's laboratory at the time, and in particular on whether Popovic grew LAV in continuous rather than transient cell cultures. As it happens, it was Crewdson's attempts to resolve questions of this type that led him to argue that HTLV-IIIB was derived from LAV rather than an independent isolate.

Once again though, a final answer may be impossible to obtain, largely because many records, especially those kept by Popovic, no longer exist. "The real problem is that Popovic never kept a notebook, period," says Gallo. (Popovic was in his native Czechoslovakia last week and could not be reached.) Given the increasingly rigid standards for laboratory records being set as a result of other notorious misconduct cases, "the missing data could be a problem" in the investigation, says Gallo. "We are in new times."

In his statement, Raub announced that the NIH Office of Scientific Integrity which had handled the inquiry, would also undertake the formal investigation with a yet-unnamed panel of "expert scientific advisors" and the continued oversight and guidance of an 11-person panel nominated by the National Academy of Science (NAS).

Missing data

This summer, the NAS panel wrote to Raub, saying that they had found that "some data appears to be missing from the data books . . . [and] there is a possibility of selection and/or misrepresentation of data." They requested that the inquiry be terminated and a full investigation begun at that time. But Raub declined, explaining that "redesignation now would be premature" given that the "inquiry is in an especially significant phase". But what, exactly, was learned between that time and last week remains unclear.

Gallo says the announcement caught him by surprise both by its timing and its conclusion. "I expected this to be finished long ago. I think the major questions of anybody taking anything is over." One of the key allegations raised by Crewdson was that Gallo attempted to take full credit for the discovery of the AIDS virus even after he was familiar with Montagnier's work. Gallo acknowledges that investigators may still conclude that his papers show "exaggeration in the interest of petty gain", but that is an interpretation he believes is due to sloppy paper-writing in a tense period. "It was an emergency time. We weren't worried about dotting the 'i's and crossing the 't's" he says.

Representative John Dingell, chairman of the Energy and Commerce investigations subcommittee that asked NIH to undertake the inquiry, said in a statement that "we intend to follow [NIH's] investigation closely, and will also give them an opportunity to explain to the subcommittee why they have chosen not to pursue all of the allegations" that were most throughly aired in Crewdson's article last year.

Christopher Anderson, David Concar & Alun Anderson

INTERNATIONAL COLLABORATION

Ulysses treads a lonely path

London

After a successful launch and deployment from the space shuttle Discovery on 6 October, the Ulysses solar probe is now speeding towards Jupiter on the first leg of a five-year voyage to fly over both poles of the Sun. The mission is still a collaboration between the European Space Agency (ESA) and the US National Aeronautics and Space Administration (NASA), even though a bitter taste remains from NASA's sudden cancellation of a companion probe to Ulysses in 1981.

Ulysses will be the first spaceprobe to fly over the Sun's poles, taking measurements of the solar wind (see *Nature* **347**, 439; 4 October 1990), its associated magnetic field patterns and the Sun's output of X-rays, radio and plasma waves. Until now, all solar observations have been made from the same ecliptic plane in which the Earth orbits the Sun. Spacecraft launched from Earth are flung out in this plane automatically, because of the Earth's own movement, and there is no launch vehicle sufficiently powerful to put Ulysses directly into a solar polar orbit. To overcome the problem, Ulysses will make a 'gravity assist manoeuvre' around Jupiter in February 1992 (similar to those made by Voyager 2 during its tour of the outer planets), stealing energy from the planet to send it in a southern trajectory back towards the Sun, culminating in a south polar flypast in the summer of 1994. Ulysses will then swing round the Sun, flying over the north pole one year later.

Richard Marsden, ESA deputy project scientist, is enthusiastic about the complement of instruments flying on Ulysses, picking out the measurement of heavy element ions in the solar wind and the detection of cosmic rays from outside the Solar System as particularly promising experiments. But Marsden says the loss of the NASA probe will cause "certain problems in interpreting the data".

In the original plan, two probes would have flown past opposite poles simultaneously, allowing both to be studied at the same time with a core payload of instruments shared by the two craft. Ulysses will observe both poles, but it will now be difficult to tell if observed differences between the north and south poles are due to inherent differences between the two or to variation in the Sun's activity in time. Observations using an X-ray/extreme ultraviolet telescope and of the Sun's corona were to be made from the companion craft and are now lost completely.

Joan Johnson-Freese, from the Center for Space Policy and Law at the University of Central Florida, says that NASA's contribution to what was then known as the International Solar Polar Mission (ISPM) was the victim of a "unique set of circumstances". In 1981, President Ronald Reagan's new administration was making deep cuts throughout the US budget, while NASA was stripping resources from other space programmes to meet the space shuttle's ballooning costs. NASA was told that one project from the ISPM, the Hubble Space Telescope or the Galileo Jupiter probe, would have to be cut.

But it was not NASA's cancellation of the second ISPM probe that angered ESA officials so much as the lack of consultation before the announcement. Johnson-Freese says ESA and other collaborators were left asking themselves the question: "what good is a memorandum of understanding?", if US budgetary squeezes can jeopardize collaborative projects. She says this has coloured recent negotiations on collaborations such as the planned Freedom space station and the Earth Observing System remote-sensing network.

Ian Pryke, head of the ESA office in Washington, maintains that ESA's relations with NASA are now back on course after the "low point" of the early 1980s. But ESA has little choice but to collaborate with NASA on expensive space science projects, given the limits of its own science budget. Ironically, Willis Meeks, NASA project manager for Ulysses, says the savings achieved through the cancellation were "probably nil". NASA is still paying for two-thirds of the $750-million mission, supplying the expensive shuttle launch, the booster rocket to power Ulysses towards Jupiter and use of its Deep Space Network. Any savings have been dwarfed by cost overruns because of repeated launch delays.

The original launch date was February 1983, but NASA was forced to delay by two years in 1981, to allow that year's budget to balance (despite the fact that this increased overall cost), and the 1986 Challenger disaster caused further setbacks.

Johnson-Freese says ISPM was the victim of the US budgetary system, where government spending is determined year by year. This means that agencies such as NASA and the National Science Foundation (NSF) cannot guarantee a continued level of funding for their side of agreed international 'big science' projects.

D. Allan Bromley, science adviser to the US president, has stressed the foreign perception of the United States as an "unreliable partner" in many of his speeches, and called for "something more like treaty arrangements" to govern international projects.

One solution would be longer-term earmarking of funds for important collaborations. But Dick Malow, a member of staff of the House of Representatives appropriations subcommittee that handles the NASA and NSF budgets, says the US presidential system of government, where members of the House of Representatives are re-elected every two years, precludes this change. Other congressional staff turn criticism of the US budgetary system back on NASA, claiming that NASA has repeatedly given low cost estimates for 'new start' projects and then expected Congress to pay for cost overruns. The same staff say that NASA is still guilty of a lack of realism, asking for an annual budget growth of 15 per cent in real terms in the face of the huge current US budget deficit. **Peter Aldhous**

GENETIC ENGINEERING

Glasnost for UK release information

London

Bowing to pressure from environmentalist groups and members of the House of Lords, the UK government is to amend the Environmental Protection Bill so as to ensure public access to information on proposed releases of genetically engineered organisms. The government had originally argued that clauses on public access were not needed because the Environment Secretary already has the power to make this information public.

The new clauses propose public registers containing applications for consents to release, supporting information supplied with these applications, and any advice given to the Environment Secretary by the new independent Advisory Committee on Releases to the Environment (ACRE). Some information may be withheld if the Environment Secretary decides that this would undermine national security, provoke sabotage by extremist groups which would present an environmental hazard, or breach commercial confidentiality. But in commercially sensitive cases, the name and address of the applicant, a description of the organism and the purpose of the release, and the results of any assessment of the environmental risks posed must still be included.

Julian Kinderlerer, from the University of Sheffield, a member of ACRE who has been advising the opposition Labour party on the release of engineered organisms, says he is "thrilled" by the amendments, and surprised at how far the government has gone. The publication of ACRE's advice is particularly significant, he says, because it puts the onus on the Environment Secretary to justify any decisions that go against ACRE's advice. A further amendment makes ACRE a statutory committee, so that a decision of parliament would be needed to abolish it or alter its status. The new amendments will be discussed in the House of Lords next week, at the bill's report stage. Peter Aldhous

ILLEGAL EXPORTS

Pass the gas masks, please

Munich

NEW revelations that German companies have helped virtually every area of Iraqi military development, including its chemical weapons programme, are continuing to rock the government of Chancellor Helmut Kohl. Rather than shipping weapons themselves, it now appears that German companies concentrated on exporting seemingly harmless goods or machinery that could easily be used to make weapons. Among the allegations are that German companies built nerve and mustard gas factories, helped Iraq to enhance its nuclear and biological weapons programmes, built factories for advanced conventional weapons and helped in the attempt to construct the infamous 150-metre-long giant cannon.

In the latest development, a manager and an engineer from two West German companies confessed last week that they had known that they were helping Iraq to build factories that can be used to produce poison gas. Previously they had contended that the factories were to be used for pesticide production (see *Nature* **332**, 573; 1988).

The West German government acknowledged the huge range of shipments in a secret briefing given by Economics Minister Helmut Haussmann to the Economics Committee of the Bundestag in August. According to one committee member, Haussmann admitted that the range of exports was broader than previously suspected and that West German laws would not necessarily be able to prevent future exports, especially of so-called 'dual-use' technology.

Oskar Lafontaine (Social Democrat), who is challenging Kohl (Christian Democrat) for the post of chancellor in the December elections, declared in a speech before the first meeting of an all-German parliament on 3 October that "it is our responsibility to change the German weapons export policy. We in a new, larger Germany cannot go on [selling] first the gas to Iraq, then the gas masks to Saudi Arabia . . . ." West Germany came under severe criticism from the United States last year for its export to Libya of a poison gas plant. The export embarrassed Bonn and caused it to strengthen the laws regulating export of weapons technology, as well as to pursue violators more aggressively. German law is now stricter than that of other European countries and prosecutors are investigating more than 170 companies in 37 separate proceedings, according to the news magazine *Der Spiegel*.

But it took pressure from the opposition Social Democrats to give the new laws any bite. As passed by the Christian-Democrat-controlled Bundestag, the amended laws would not have increased above one year the minimum criminal penalty for violations. The Bundesrat (upper house), which is now controlled by the Social Democrats, forced minimum penalties of two years and more.

The difference is significant, according to Michael Brzoska, an expert on arms control at the University of Hamburg. Traditionally, he says, defendants given one-year sentences are released on probation by German courts. Public prosecutors normally do not expend the tremendous effort necessary to make a case against exporters unless they expect the defendant, if found guilty, to go to prison.

Brzoska, who has written several books on arms exports, says that Bonn deserves its bad reputation even though other countries, including France and the Soviet Union, exported more weapons to Iraq. Given the size of its economy, he says, "[West] Germany has been proportionally no worse than other countries", but it has made itself conspicuous by ignoring the problem of illegal or dual-use exports. The export authorities did nothing, he says, when clues poured in concerning "hundreds of cases".

Even after the changes in the law, Brzoska says that Germany could be doing a lot more to stop dubious exports. Industry is often given the benefit of the doubt, he says, because "the export laws are written so that business should be disturbed as little as possible." Instead, Brzoska says, the laws should be rewritten so that everything is checked that might be useful for weapons production.

Steven Dickman

FOREIGN RESEARCHERS

Asking for trouble

Paris

A SMALL spin-off from the Persian Gulf crisis is causing trouble in the French national research organization, CNRS. First, a government directive was issued asking for the names of all visiting scientists from Iraq. Then Claude Paoletti, director of the life sciences section of CNRS, sent a letter to heads of the 300 CNRS life sciences laboratories asking for the names not just of Iraqis, but also of researchers from Kuwait, Iran, Turkey, Morocco, Algeria, Tunisia, Egypt, Libya, Syria, Jordan, Lebanon and Pakistan. The affair has now caught the eye of the Paris-based League for Human Rights, and Paoletti's action, apparently taken on his own initiative, has brought more opprobrium to an already controversial character (see *Nature* **339**, 326; 1989).

According to Rose Katz, secretary general of SNCS-FEN, the major trade union representing researchers, two Iraqi scientists have already been asked to stay at home. One, a doctoral student in physics, was working on sensitive aerothermal techniques at Meudon, near Paris. The other is a researcher at the national agricultural research institute.

What has particularly irked the union and civil rights bodies is Paoletti's apparently personal decision to extend the scope of the government's request. SNCS-FEN was quick to denounce his request as "discriminatory" and to ask its members to ignore the letter.

Meanwhile, the League for Human Rights has opened a dossier on the affair and has requested the national commission on the freedom of information (CNIL) to investigate the legality of CNRS's action in obtaining and filing the information requested. At the end of September, the director-general of CNRS, François Kourilsky, issued a statement denying accusations of racism, but leaving Paoletti to fend for himself. "Our concern was only to contact Iraqi researchers", says Kourilsky, who added that the request of the life sciences section to update its list of foreign students was "badly conceived in the current climate of the Gulf crisis". But he said there was no discriminatory intent on Paoletti's part.

Paoletti would not comment last week. But at CNRS headquarters in Paris, Jean-François Huchet, reponsible for external relations, confirmed that Paoletti's request for details of visitors from other Muslim countries was routine practice, albeit ill-timed. "The files we keep on researchers have all been authorized by the CNIL", said Huchet. "Dr Paoletti anticipated a little, but we have to keep these files up to date." Huchet said that under special circumstances, such as the Gulf crisis, or the massacre of Chinese students in Beijing, the administration may ask for a head-count of students potentially implicated. "I think this is good management", says Huchet. "If we did not know where visiting students are or what there status is, we would be accused of sloppiness".

Some directors of life sciences laboratories have followed union advice and ignored Paoletti's letter. "If he wants to check up on foreign students he should call in the police", said one laboratory director — a reaction that Huchet called "professional misconduct".

Meanwhile, the president of the League of Human Rights is still not satisfied with Kourilsky's explanation and is waiting for the CNIL to give its opinion on the legality of CNRS files.

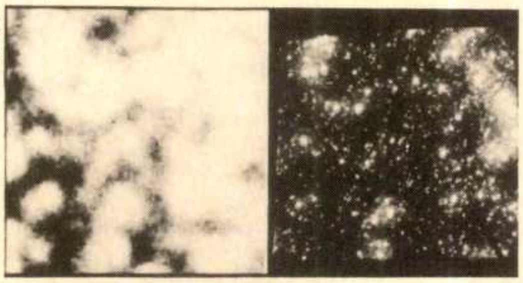
Peter Coles

HUBBLE SPACE TELESCOPE

A pictorial progress report

Baltimore

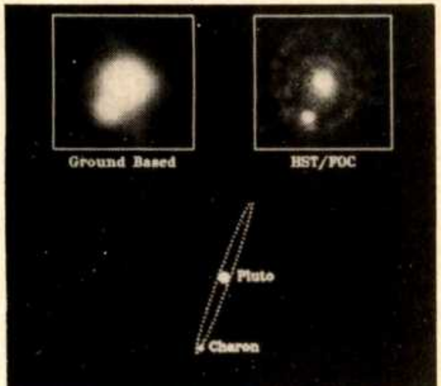
SINCE the days of gloom and pessimism that immediately followed the discovery of spherical aberration in the optics of the Hubble Space Telescope (HST), astronomers have become more cheery as they have learned more about what the HST can still do. Last week, during a show put on at the Space Telescope Science Institute in Baltimore to display the latest achievements of the Wide Field/Planetary Camera (WFPC) and Faint Object Camera (FOC), HST director Riccardo Giacconi declared that he was "cheerfully depressed": the telescope has obvious limitations, but in certain applications it is nevertheless far superior to ground-based telescopes, as the images on this page demonstrate.


NASA/ESA

In 1938 a nova exploded in the globular cluster M14, and in the photograph (left) a faint star, near the centre of the image, was found which was of unusual colour. It was tentatively identified as the nova in its now quiescent state. But the FOC picture (right) (so much higher in resolution that corresponding images in the two photos can be identified only with difficulty) dispels this idea. In place of the faint nova, as many as a dozen candidate stars appeared, all of normal colour. The unusual colour seen from the ground was due to a conspiracy of ordinary stars getting together to look like one odd one.

The key to recent progress has been what some HST scientists call the D-word: deconvolution. HST's optical flaw spreads each point source of light in an image into a blur of known profile. Deconvolution is the mathematical procedure by which the blurring is unfolded from the images to create an 'unblurred' photograph (see *Nature* **346**, 686; 1990).

This process can approach perfection for images that consist of a small number of isolated point sources — a loose grouping of stars, for example. But for dense star clusters, where the individual images overlap, and even more for nebulous extended objects whose brightness varies continuously over a large area, deconvolution is less successful; it cannot put back into an image information which the blurring has destroyed. Among last week's presentations, a few outright failures were included. Sandy Faber, of the Lick Observatory in California, concluded after taking some test images that the HST in its present form would not be able to perform one of its most eagerly awaited tasks, that of pinning down the expansion rate of the Universe. An essential step in this measurement was to have been the detection of Cepheid variable stars, whose intrinsic brightness can be deduced from their period of variation, in distant galaxies. Comparison of the measured brightness with the deduced intrinsic brightness then yields an accurate distance with which to calibrate the scale of the Universe. But, as Faber discovered, the brightness of faint Cepheids in distant galaxies cannot be measured with sufficient accuracy on the blurred images, and deconvolution degrades brightness information as it restores spatial resolution.



A simple demonstration of resolving power. The right-hand FOC photograph of Pluto and its large satellite Charon is a "raw" image, with no attempt made to remove the clearly visible halos due to spherical aberration, but is still a clear improvement over the best ground-based image (left).

HST astronomers are now engaged in a science assessment programme (during which the images shown here were obtained) whose purpose is to characterize what the instruments can do, and what they cannot. These findings will be distributed to the astronomers currently on the roster of scheduled observing time so that they can decide whether their planned observations can be done, perhaps with longer exposure times, or if they should be postponed until the HST has been fixed. Early next year, the Telescope Allocation Committee will reconvene to decide on a new schedule of observations.

Despite the spherical aberration, and despite the continuing jitters that still afflict HST when it crosses from day to night and back again, astronomers are clearly happy with what they have done, and will be able to do. Last week's presentation of new images was unabashedly an attempt to show that HST can still do wondrous things. It succeeded.

David Lindley

RESEARCH LOBBIES

New research lobby draws fire

Washington

FOUR congressmen last week announced the formation of a 24-member legislative caucus aimed at promoting basic biomedical research and held its first meeting, a seminar on maintaining the United States' leading position in the field. But although the seminar attracted such luminaries as Nobel Laureate Harold Varmus of the University of California and Leon Rosenberg, dean of the Yale University School of Medicine, some leading members of Congress were absent.

Most prominent of the names absent from the caucus lineup is William Natcher (Democrat, Kentucky) chairman of the House of Representatives appropriations subcommittee that controls most of the federal biomedical research funding. Other missing research supporters include full appropriations committee chair Jamie Whitten (Democrat, Mississippi) and minority chair Silvio Conte (Democrat, Massachusetts). "It's surprising to see a biomedical research caucus that doesn't have any of the long-term research champions, especially since these champions already think of themselves as a caucus", says Carol Sheeman of the American Association of Universities.

Christopher Anderson

Industry fights back

Washington

US BIOMEDICAL companies and associations are planning to put together a new organization to oppose the public relations efforts of anti-animal research activists, industry officials said last week. Although plans have not been finalized, Anneliz Hannan, director of corporate affairs at US Surgical Corporation and a principal organizer of the project, says a budget of $17–$25 million a year will be needed to counter animal activist campaigns adequately. Rather than duplicate the work of existing groups that focus on legislative lobbying, the new organization will serve as an 'umbrella' group, she says.

The new organization will target high schools, either by educating teachers or addressing students directly, Hannan says. An education programme now being sponsored by US Surgical in Connecticut may serve as the model. The American Medical Association, Procter and Gamble, and Squibb Pharmaceuticals are said to be among those interested in setting up the new organization. A possible director of the organization is rumoured to be Frederick Goodwin, currently the administrator of the Alcohol, Drug and Mental Health Administration and an outspoken advocate of animal research.

Christopher Anderson

CONTRACT RESEARCH

Industry gets a bargain

London

BRITISH universities, trying to survive by taking on an increasing number of industrial research contracts, may be undermining themselves instead by failing to charge for overhead costs in full, according to the author of a new report* on British research spending.

Peter Collins and colleagues from the Science and Engineering Policy Studies Unit (SEPSU), run by the Royal Society and the Fellowship of Engineering, have completed the first survey to identify the detailed structure of research spending in a sample of British universities, and government and industrial laboratories. Collins' stark warning on university research contract pricing policy follows a Science and Engineering Research Council (SERC) survey showing that British spending per researcher in higher education institutes lags behind that in preunification West Germany by 40 per cent.

The SEPSU study looked at research spending in 30 laboratories and university departments in 1986–87, separating salaries from all other costs (including equipment, travel, administration and laboratory maintenance). In the universities, these costs varied between 80 and 140 per cent of the salary bill. In industry, this figure rose to between 165 and 240 per cent.

According to Collins, these data show that researchers in industry are, "project for project, better equipped" than their academic counterparts. But he adds that the problem of maintaining a 'well found' university laboratory is compounded by the consistent failure of universities to recoup the the full indirect costs of research contracted out from industry.

The old University Grants Committee (now replaced by the Universities Funding Council, UFC) assumed that research project overhead costs are about 40 per cent of the direct costs supplied through a research council grant or industrial contract. Collins says this figure is far too low. Even so, universities on average recovered only 14 per cent of the value of external research contracts to cover overheads in 1988–89, giving the lie to recent accusations from industry that universities are overcharging (see *Nature*, **347**, 216; 20 September 1990). With these external contracts increasing by nearly 14 per cent per year throughout the 1980s, Collins says universities will be "in trouble", if they do not soon start charging a proper price for their services.

Miles Hedges, the UFC's financial adviser, says the UFC is encouraging vice-chancellors to increase their research contract prices. He senses some improvement, but adds "there's an awfully long way to go". Sue Jacobs, from the industrial liasion group at Imperial College London, which has been more successful than most at recovering its overheads, says that (with a few exceptions) industrial companies can be persuaded to pay a fair price for university research. But she finds getting a fair deal from government departments more difficult. The problem, she says, is finding the right government official to negotiate with.

The new SERC survey is the first to calculate research spending per full-time researcher in British universities. It shows that West Germany spent £80,000 per academic researcher in 1987, compared with £48,000 in the United Kingdom. German academics are better equipped, have more support staff and are better paid than their British counterparts, the report concludes.

British spending seems to compare favourably with that in France (£36,000 per full-time university researcher). But Harry Atkinson, one of the report's authors, says that this is misleading: French research spending is biased heavily towards the laboratories of the Centre National de la Recherche Scientifique (CNRS), many of which are associated with universities.

If links between universities and CNRS laboratories are taken into account, Atkinson says, French university spending is probably similar to that in the United Kingdom.

Peter Aldhous

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* The structure of research expenditure, SEPSU Policy Study 4, obtainable from SEPSU, Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.

ASTRONOMY

Mt Graham sites cleared

Washington

ALTHOUGH its legal battles are far from over, the University of Arizona has put behind it one of the chief barriers to the construction of the controversial Mount Graham telescopes. After a three-judge panel gave the go-ahead last week, the university quickly felled trees to clear space for the first two telescopes on the sites. Over the protests of environmentalists, 24 of whom were arrested, workers began digging trenches on the sites. With just weeks left to go before the approach of winter stops further work, the construction is largely a symbolic gesture to defuse further lawsuits and to reassure the telescope's German and Vatican partners that progress is being made.

Activists plan to continue the legal battle which has already been running for three years and involved innumerable court hearings (see *Nature* 344, 479; 1990).

Christopher Anderson

MUSEUM POLICY

Directors agree on research

London

No research, then no museum. That was the theme of an international conference on the future of scholarship in museums held last week (2 October) by the Royal Society of Arts. The message was notably spelled out by Professor Wolf-Dieter Dube, director of the State Museums of the Prussian Cultural Heritage in Berlin.

Continuing controversy over research posts at the Natural History Museum (NHM) in London spilled over into the meeting, which had been arranged long before the NHM crisis had erupted. The museum's director, Dr Neil Chalmers, contributed to a round-table discussion at the end of the meeting.

Also running through the discussion was the general feeling that museums everywhere are common property, and that their managers should maintain the expertise necessary to interpret collections. And museums must convey the essentials of new research to the public without patronizing people.

The Science Museum in London claims to follow the second precept. Popularization, said director Dr Neil Cossons, needs the support of "high-grade scholarship" to make it worthwhile. But he also noted that museums have outgrown the ability of old-fashioned academic museum managers to cope either with themselves or public demand. Victims of their own success, "museums in the past quarter-century have moved from the twilight to the spotlight". Scholars, urged Cossons, must take part in management, and not regard it as "a pollution of the purity of scholarship".

The sheer pace of change has led to confusion. Dr Klaus Sattler, a German researcher currently working at the NHM, commented that compared with the management at the NHM and its neighbour, the Victoria and Albert Museum, "the total collapse of my country in 1945 looks positively well-organized".

Responding to questions about the London NHM's corporate plan, Dr Michael Novacek, dean of science at the American Museum of Natural History, said he was disturbed at the way in which decisions on priorities had been made and that NHM staff were apparently not consulted about future research directions or warned of the dangers of "jumping on biodiversity bandwagons".

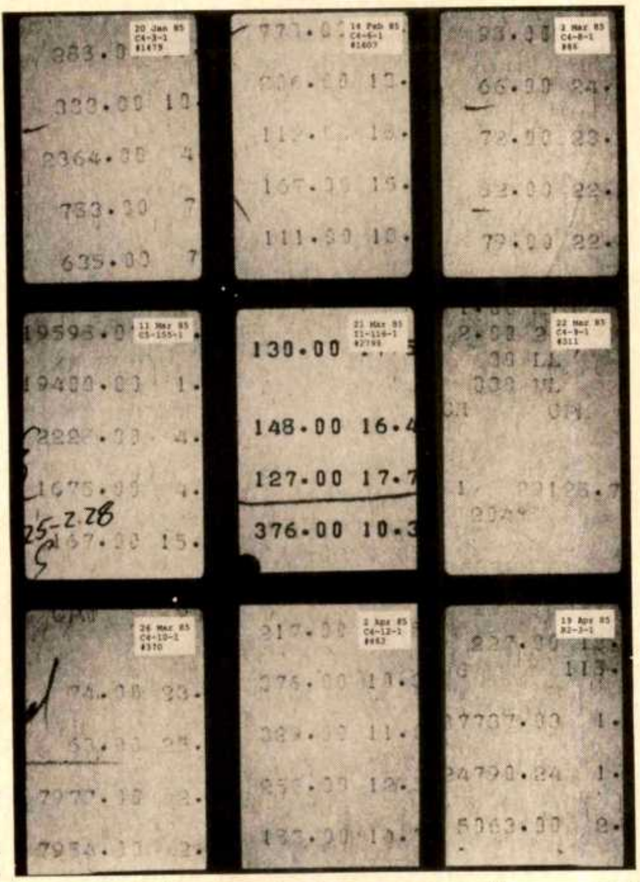
Henry Gee

SCIENTIFIC MISCONDUCT

Science meets forensic science

Washington

THE photograph below, an example of evidence from the continuing misconduct investigation of Tufts University immunologist Thereza Imanishi-Kari, shows what the detective techniques of forensic science can do to analyse laboratory research records.



Faced with the problem of dating Imanishi-Kari's records to determine if fabrication occurred in the preparation of a 1987 *Cell* paper (see *Nature* 347, 317; 1990), the staff of the congressional investigatory subcommittee of Representative John Dingell (Democrat, Michigan) focused on a series of gamma-counter tapes in the notebooks. They noted that the print quality of the tapes declined markedly as the machine's ribbon got older. On some of the tapes the staff also found a cumulative number stamp.

Joined by the Secret Service, the subcommittee staff decided to compare these tapes with tapes from notebooks of other researchers in the Massachusetts Institute of Technology laboratory where Imanishi-Kari had worked.

In Boston, they found that several other researchers and students had kept records of counter tapes from the 1985 period covered by Imanishi-Kari's notebooks. The investigators zoomed in on one of the tapes that came during a key part of Imanishi-Kari's experiments and, most importantly, still displayed its cumulative counter number. Around it they placed tapes from other researchers taken during the same period as the date marked in Imanishi-Kari's notebook.

When the investigators compared the tapes, as in this photograph (made available to *Nature* by the subcommittee), they immediately noticed the central one — Imanishi-Kari's tape — obviously stands out of chronological order. In that tape, the counter clearly had a new ribbon (the tape dated the next day is from a very old ribbon) and the counter number is badly out of sequence. Only one counter accessible to Imanishi-Kari had this characteristic printing style (two zeros after the decimal and so on) and font. Based on this sort of evidence, the Secret Service concluded that the date on the tape is off by many months. Whether that suggests falsification of data is, of course, a completely different matter.

Among the other tools the Secret Service uses in such cases are hand-writing, chemical, microscopic and even neutron-activation analysis to find not only where paper came from, but when and where it was used. They can also determine, for example, if two apparently concurrent data sets actually came from the same paper roll or notebook.

Christopher Anderson

UK BIOTECHNOLOGY

Biotechnology no longer Wellcome

London

WELLCOME Biotechnology, since 1982 a separate division within the British pharmaceutical company Wellcome, has become the chief casualty of Wellcome's defeat by the US biotechnology company Genentech over the right to market tissue plasminogen activator (TPA) in the United States (see *Nature* 344, 692; 1990).

Salvador Moncada, director of research for the Wellcome Foundation, says that the loss of the valuable US market for Wellcome's TPA, together with the cessation of contracts to manufacture animal vaccines in 1992, means that the biotechnology division has lost its economic *raison d'etre*. It will be wound up over the next few years.

The remaining products from Wellcome Biotechnology (Wellferon, an alpha-interferon, and Campath, an antibody-based anti-cancer agent) and some biotechnology research will be integrated into other sections of the company. Moncada says that the restructuring will involve a reduction in the "head count" of researchers, but he says that the company is is looking for ways to retain as many as possible of the division's 100 or so researchers. Decisions on which research projects are to be retained will be taken in the coming weeks.

The closure of Wellcome Biotechnology is part of a restructuring that includes Wellcome's withdrawal from human vaccine production. News of this has generated considerable criticism, as Wellcome is the single remaining large-scale producer of vaccines in the United Kingdom, and an important supplier for the National Health Service (NHS). But the company says that production will continue until the NHS has found alternative suppliers.

Peter Aldhous

NOBEL PRIZE

Transplantation wins again

London

IN a surprise decision, the Nobel Prize for Medicine has been awarded to Joseph E. Murray, from Brigham and Women's Hospital in Boston, and E. Donnall Thomas, from the Fred Hutchinson Cancer Research Center in Seattle, for their work on human organ and cell transplantation. Murray carried out the first successful kidney transplant between identical twins in 1954 and later pioneered the transplantation of kidneys from deceased donors and the use of whole body irradiation to induce immunosuppression.

In the late 1950s, Thomas managed to diminish the severe 'graft versus host' reaction in bone marrow transplants and showed that transplanted cells can repopulate the host bone marrow.

The award is the third in the field of transplantation in the past 30 years, following Sir Peter Medawar and Sir MacFarlane Burnet's 1960 prize for discovering immunological tolerance and the 1980 award to Jean Dausset, George Snell and Baruj Benacerraf for the discovery of HLA human histocompatibility antigens. This year's prize is controversial, as it can be argued that other researchers working at about the same time made equally important contributions to the development of transplantation as a clinical technique, notably two Frenchmen, Jean Hamburger and Georges Matté. Hans Wigzell, chairman of the Nobel Committee, says it is "more difficult in the clinical world [than in basic science] to identify pioneers".

Peter Aldhous

RADIATION EXPOSURE

Sellafield under scrutiny

London

THE UK Health and Safety Executive (HSE) plans to extend the survey which earlier this year suggested a link between the high incidence of leukaemia in children in the West Cumbrian village of Seascale and their fathers' exposure to radiation at the Sellafield (formerly Windscale) nuclear reprocessing plant (see *Nature* **343**, 679; 1990).

The new HSE study, which will cover everybody who has worked at Sellafield since 1949, is one of a number of projects designed to investigate the controversial link between paternal radiation exposure and childhood cancer originally suggested by Professor Martin Gardner of the University of Southampton. These studies should soon begin, once official clearances have been obtained and problems with data confidentiality resolved.

The HSE study will compare the full work histories of the fathers of leukaemic and unaffected children, looking at other factors besides radiation dosages alone. Exposure to carcinogenic chemicals, for example, has been put forward as an alternative explanation for Gardner's results. Any Sellafield workers wishing to have their records excluded from the study must get in touch with the HSE by the end of November.

Gerald Draper, from the University of Oxford, plans a wider study, linking Oxford's National Registry of Childhood Tumours with the National Radiological Protection Board (NRPB)'s radiation dose records for all British radiation workers. But Draper says progress has been "snarled up" because of the confidentiality of vital data held by the Office of Population Censuses and Surveys (OPCS).

To link the two databases, Draper needs to know the date of birth of the fathers of leukaemic children, to check that matches between the names of fathers and workers on the NRPB database are genuine. A 1938 act of parliament allows only OPCS staff access to these data.

Draper says a compromise should be possible, with OPCS staff doing some preliminary analysis and providing the Oxford team with summary data, so that individuals cannot be identified. He now hopes to get approval for the study within the next few months.

If the 'Gardner effect' is real, and the risk factor is anything like as large as Gardner suggested (a seven to eightfold increase in the chance of fathering a leukaemic child for fathers receiving a total radiation dose of 100 milliSieverts, or more than 10 mSv in the six months before conception), Draper says his survey should be large enough to detect the effect.

In Scotland, where population records are subject to a different law, data confidentiality has not been a big problem. But studies have still been held up by the need to get official approval from the HSE and the Department of Health.

The Committee on Medical Aspects of Radiation in the Environment, independent scientific advisers to the UK government, said that further study following the Gardner report should be co-ordinated centrally to avoid unnecessary intrusion into the lives of British radiation workers (see *Nature* **344**, 576; 1990). This was intended to avoid duplication of intrusive studies requiring questionnaire data, but has also held back those simply involving the analysis of existing records.

Naturally, we have a contingency plan— another name change
NEWS
LEUKAEMIA SURVEY EXTENDED AT SELLAFIELD
BIRCH

Leo Kinlen, from the Cancer Research Campaign's laboratories in Edinburgh, last week received approval for a study that will link records of Scottish leukaemia cases and controls with radiation records for the entire Scottish nuclear industry. He hopes to complete arrangements for access to the industry's radiation dose records within the next few weeks. A second study is planned by the Scottish Health Service, but both Scottish surveys may be too small to give a definitive answer on the existence of the Gardner effect. Nevertheless, Kinlen says the studies should give an indication of the "size of the problem [in Scotland], if problem there is".

Two surveys commissioned to investigate the smaller excesses in leukaemia incidence near nuclear sites at Dounreay in Scotland, and Aldermaston and Burghfield in southern England, are now nearing completion. The Dounreay study should be published within the next few months, the Aldermarston and Burghfield study before the end of 1991.

Peter Aldhous

- Researchers from AEA Technology show that plutonium-239 does not accumulate in large quantities in human testes. This has been put forward as a mechanism to explain the Gardner effect. See Scientific Correspondence page 521.

RESEARCH IN JAPAN

Rent-a-lab welcome for foreigners

Tokyo

JAPAN'S Ministry of International Trade and Industry (MITI) is opening up some of its major research programmes to foreign participation and making some of its best-equipped facilities available for rent in a campaign designed to counter foreign complaints that the Japanese research system is closed to outsiders.

At the end of last month, MITI announced that three huge programmes run by the New Energy and Industrial Technology Development Organization (NEDO) are now open to foreign participation. The programmes are in advanced chemical processing technology, software development and in the design of clothes that "reflect human sensibility". But the change in policy is unikely to bring in a horde of foreign companies as it was announced only in the *MITI Gazette* and scarcely a month remains for applications to be filed.

More foreign interest may be generated from a second MITI decision, announced last week, to open some facilities for public use. For as little as ¥200,000 ($1,460) foreign companies or individuals will be able to rent a ten-second free-fall ride aboard a microgravity capsule that can be shot down a disused coal mine shaft in Hokkaido. The mine shaft at the Japan Microgravity Center is 710 metres deep and MITI claims that it provides longer near-zero-gravity conditions than at any other ground-based facility.

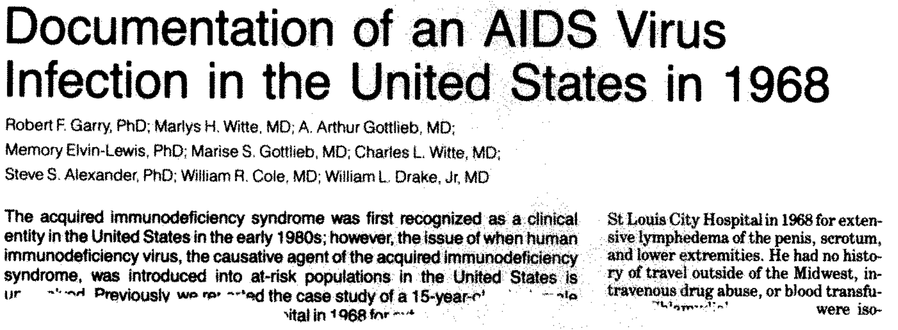
Also available for rent will be some of the world's most powerful ion beams, courtesy of the new Ion Engineering Center in Kansai science city, and the facilities of the Research Center for Industrial Utilization of Marine Organisms, the Japan Ultrahigh-Temperature Material Research Center and the Applied Laser Engineering Center.

All these institutes are run as corporations by NEDO which was reorganized in 1988 to absorb most of MITI's major research and development projects. The corporations receive half of their funding in the form of investment from NEDO, private companies and local government. Rental charges are intended to cover operating costs and not to provide a profit, says MITI. At first, the major users are expected to be the Japanese companies that have already invested in the institutes.

David Swinbanks

Early case of AIDS in the USA

SIR—Two recent news items in *Nature*[1,2] highlight the case history of a 25-year-old seaman who died in Manchester, England, of *Pneumocystis carinii*, and in retrospect, of human immunodeficiency virus (HIV) infection, in 1959, the same year as the earliest serologically confirmed case of AIDS in Africa. The polymerase chain reaction (PCR) was used to amplify HIV genetic material in stored tissue specimens, although distinction between HIV-1 and HIV-2 was inconclusive[3]. Contrary to a statement in one of the *Nature* articles[1], it is not known whether the Manchester seaman spread his HIV infection[4]. However, the other commentary[2] refers to another report of serologically confirmed HIV-1 infection in a Norwegian family including a father (the index patient, also a seaman, whose multisystem illness began in 1966), his wife, and daughter, all of whom died in 1976[5].



Documentation of an AIDS Virus Infection in the United States in 1968

Robert F. Garry, PhD; Marlys H. Witte, MD; A. Arthur Gottlieb, MD;
Memory Elvin-Lewis, PhD; Marise S. Gottlieb, MD; Charles L. Witte, MD;
Steve S. Alexander, PhD; William R. Cole, MD; William L. Drake, Jr, MD

The acquired immunodeficiency syndrome was first recognized as a clinical entity in the United States in the early 1980s; however, the issue of when human immunodeficiency virus, the causative agent of the acquired immunodeficiency syndrome, was introduced into at-risk populations in the United States is [illegible] Previously we [illegible]ed the case study of a 15-year-[illegible] [illegible]ital in 1968 for [illegible]

St Louis City Hospital in 1968 for extensive lymphedema of the penis, scrotum, and lower extremities. He had no history of travel outside of the Midwest, intravenous drug abuse, or blood transfu- [illegible] were iso-

Antecedent to these reports, we documented HIV-1 infection in a 15-year-old black male admitted to St Louis City Hospital in 1968 with extensive genital and bilateral lymphoedema and systemic chlamydial infection, who died less than a year later with a progressive cachexia and disseminated Kaposi's sarcoma[6-8]. In contrast to the two well-travelled sailors cited above, our patient had never ventured outside the St Louis area. Western blot analysis of frozen serum and antigen capture enzyme-linked immunoassay of stored tissues confirmed infection with HIV-1[8]. Proviral DNA has recently been detected in his tissues by PCR[9] in collaboration with J. Sninsky and S. Kwok (Cetus Corporation, Emeryville, California) but nucleotide sequence analysis is not yet complete.

Rather than representing the initial incursions of HIV into Britain, Norway and the United States, these documented AIDS patients from the pre-AIDS era support the contention that sporadic cases occurred in developed countries at least several decades before AIDS was formally recognized in the United States in the early 1980s. Sequence analysis of proviral DNA from these patients should be of interest to elucidate relationships to current HIV-1 and HIV-2 strains and thereby help clarify the epidemiology and viral evolution leading to the AIDS pandemic.

ROBERT F. GARRY

Department of Microbiology and Immunology,
Tulane University School of Medicine,
New Orleans, Louisiana 70112, USA

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2. Concar, D. *Nature* **346**, 95 (1990).
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Science sells its soul

SIR—There is a crisis in science, particularly in the biomedical field and particularly in the United States, resulting from a new and spreading perception of the scientific endeavour. The profession of science has shifted from its basic underpinnings. It used to be, and still is in some degree, that there was an allure, a challenge, an adventure of the mind in the solution of riddles. Many were willing to work long hours, even with little pay, to make a discovery and to be recognized for the discovery.

But this has all been changed, particularly in the past decade, the 'me too' decade, for added on has been the allure of money. The thought of many established investigators has become "why should others gain large profits from my work; why should I not also cash in on it?" So there has evolved a scramble to make arrangements, to seek deals, to make sure that one will still enjoy scientific work while raking in the cash. Postdoctoral fellows and students cannot fail to notice this behaviour of their mentors, and to be influenced by it. The adventure is still sweet, but a monetary component has been added.

It used to be that a scientist worked in a university or medical school to achieve the goals of the institution — teaching and research. Now he or she is asked or cajoled to perform another function, that of bringing in money to the university. If there are no arrangements with industry to bring in patent-generated money, or licensing fees, one is looked upon as an outcast, not doing one's share to keep the university viable.

Thus, increasingly, the scientific endeavour is being looked upon by young scientists as a way of making money while gaining scientific acclaim on the side. Companies have been set up by scientists to rake in the cash; the supposed rationale has been to bring desirable products onto the public market, a public benefit as a product of the research. But to other scientists, old or young, still imbued with the former ideals of science, all this is hypocrisy. Although they recognize that science cannot be amoral, that 'pure' science is a fiction, that it is influenced by the many political and social forces in our culture, they abhor the commercialization of science.

The future of scientific research is in danger not only from a decline in funding, but also from within. As idealism drains away so too does the spirit that is the adventure of scientific research, drawing to it less and less of our dedicated youth. Sinclair Lewis's *Arrowsmith* is out; biotechnology companies are in.

PHILIP SIEKEVITZ

Rockefeller University,
1230 York Avenue, New York,
New York 10021-6399, USA

Missing the joke

SIR—I would like to make a minor but important correction to an item that appeared in the Opinion section (*Nature* **346**, 398; 1990). In connection with the use of the same vehicle for carrying food and garbage, US General Accounting Office wags are quoted as quipping: "What has four wheels and fries?" Here in America the way the joke runs as I know it is: what has four wheels and *flies*?

GEORGE CRISCIONE

Communications of the ACM,
11 West 42nd Street,
New York, New York 10036, USA

Celebrating Charles Darwin

SIR—It has recently come to light that Britain has fewer public holidays than many other countries. Your readers may wish to know that we have formed a Forum to Instate a Charles Darwin Day. It seems to us fitting that Britain should have a regular public holiday to commemorate the man who first explained humanity to humanity.

Further information may be had from the undersigned.

K. R. HARRISON

7 Birchfield Close,
Liverpool L7 9LZ, UK

Which way now for Europe?

SIR—Your leading article (*Nature* **346**, 203; 1990) deriding the indiscretions of Mr Nicholas Ridley quite missed the point of what he was saying. He was not saying, as you impute, that "rigid genetic determination" assures German domination. The view that he was expressing, and which is shared by many other people (perhaps not such a minority as the disclaimers of his ideas have hastened to imply), is that in the German position lies the seat of forces which, in my lifetime, have generated three major struggles to dominate Europe. The third is clearly in progress.

Those forces are associated with its geography, compactness, social attitudes and practices, natural resource limitations, possibly a different genetic mix from that of its neighbours — I know of no "respectable biologist" who would claim it to be identical — and many other factors, some traditional, some imposed afresh on each new generation by its uniquely landlocked situation. They have not only pushed the German people into two world wars this century, but have also inspired them to behave in ways so far beyond the pale of civilized human conduct that they have outraged the whole world — a triviality that you ignore. The partition of Germany in 1945 and the emergence of two superpowers, with the (perhaps only) common objective of suppressing a recurrence of a German-led domination, has proved to be only partly efficacious in achieving this objective.

What many fear is that these forces, which have inspired the Germans to such superior economic performance, even within these postwar constraints, will, now that partition is ended, Germany unified and the superpowers down to a single, far-distant one, move the German nation to use other forms of domination in the course of time if political and economic domination prove inadequate for the German appetite. I know of no reason why a unified Germany of AD 2000 should have feelings, ideas, aspirations, hopes, resentments and fears different from a unified Germany of AD 1900 or 1930 — or why it should act differently. That the Germans have so far been contained to the expression of economic domination is no guarantee that, now free from this containment, they will be content to act as though the constraints of partition and the Warsaw pact were still in place and NATO obligations binding.

W. FRANK HARRIS

Acomb High House,
Acomb, Hexham,
Northumberland NE46 4PH, UK

SIR—By referring to Mr Nicholas Ridley's recent *Spectator* interview in which he revealed his concern about German characteristics, as an "outburst", you accuse him of being intemperate.

But this is not the main substance of your complaint. You have inferred that his concern about German characteristics indicates his belief in their *genetic* determination. You go on to express outrage that such a view could be held by grown-up people against the beliefs of respectable biologists.

First, this response is as intemperate in tone as were Ridley's original utterances, betraying a depth of feeling inappropriate to the dispassionate and disinterested posture of a scientific journal of *Nature*'s standing. Second, there is no reason to believe that Ridley believes that German characteristics are necessarily genetic in origin. Indeed, later in your article, you allow that such characteristics as can be determined (in so far as Ridley apparently fears them) are "recognizably the products of social institutions".

Whether the product of social institutions or inherited, Ridley may argue, these characteristics are equally to be feared.

It seems that you have used the Ridley "outburst" as an opportunity to express a dogmatic position in the 'nature versus nurture' argument — which argument is clearly not relevant to Ridley's fears. Thus, far from demonstrating the "primitive roots" of Ridley's views, you have used the primitive methods of guilt-by-association in aligning Ridley's views with those who believe in the influence of genetics on behaviour.

C. G. Jung's views concerning the behaviour of Germans and other groups remain deeply unpopular in some quarters as they appeared to fuel the Nazi obsession with race — in Jung's case also there is no reason to believe that he considered *inheritance* to be the mode of passing national characteristics from generation to generation.

No, the legitimate fear of identifying national or racial characteristics is that this can lead to discrimination against vulnerable minority groups. This fear should not be confused with the fear of the uprising of a traditionally assertive nation.

If there remains doubt concerning the pedigree of the perception by non-Germans of German characteristics, further reference should be made to Tacitus, whose description of German characteristics in *Germania* is very similar to the observations of some more modern commentators of Ridley's persuasion.

J. F. MACKENZIE

Royal Infirmary, Glasgow G4 0SF, UK

To go or not to go

SIR—When invited to visit colleagues in Beijing, I too asked myself (*Nature* **346**, 594; 1990) whether my visiting China would be perceived as implicit support for its present government. After ascertaining that the American Physical Society had no clear policy on individual visits to China (as opposed to attendance at officially organized conferences), I asked several of my colleagues from the People's Republic of China. Remarkably, the response to my question "Should I go?" was unanimous: all felt that individual visits, so long as they were not government-sponsored, could only benefit scientists working in China, and I was actually encouraged to make my trip. The same response was given by this university's Chinese Student and Scholar Association, which is not known for pro-Li sentiment, and which presumably is immune to any bias that might inhibit a colleague from discouraging the travel plans of another.

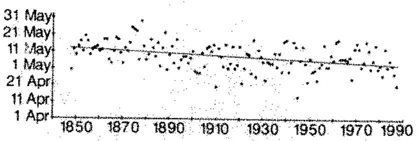
Ironically, after obtaining this 'clearance' and my visa, I was unable to make the trip. However, in the absence of new data or arguments, I would not hesitate to visit colleagues there in the future.

MICHAEL H. SALAMON

Physics Department,
University of Utah,
Salt Lake City, Utah 84112, USA

Ice-out in Maine

SIR—The folks up in northern Maine have kept an accurate record of the time of ice-out on their largest lake, Moosehead Lake, for the past 142 years. In the spring, ice-out comes suddenly, usually in one day. Moosehead Lake was the centre of the logging industry in the late nineteenth and early twentieth century, and ice-out in the spring meant fresh supplies by steamboat to the isolated logging camps on the Northeast Carry. So ice-out, like Christmas, was celebrated, remembered and luckily recorded. More recently, ice-out contests have been sponsored by the Moosehead Lake Region Chamber of Commerce. Thus, an exact record of the date of this event each year since 1848 has been preserved. When the dates are plotted and the best fit line drawn, it appears that on average ice-out occurs about 10 days earlier now than in 1848. Perhaps these data will be of interest to those studying climate change, but up here in Maine we are going to take them seriously and start reducing greenhouse gases.



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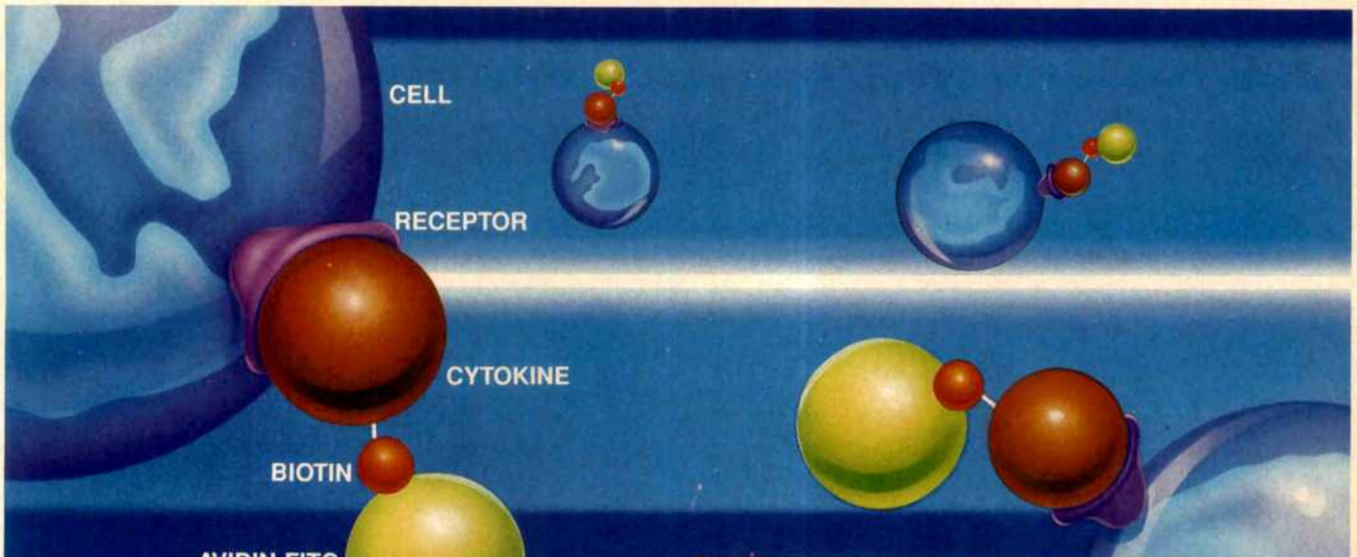

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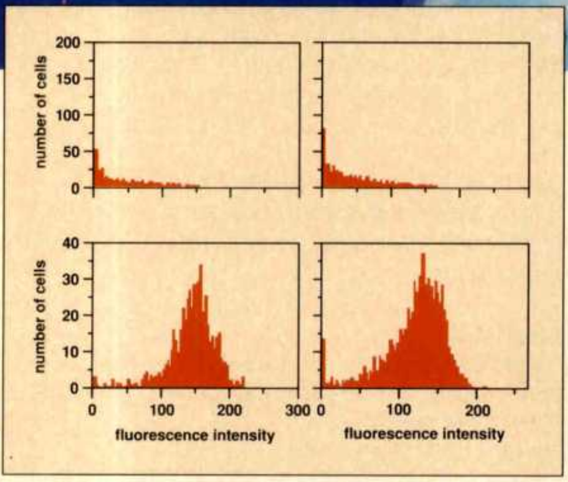

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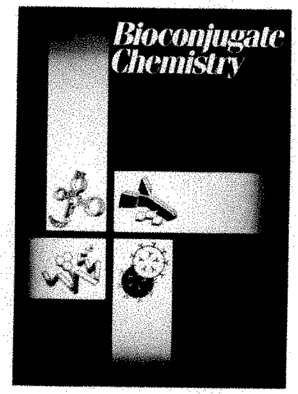

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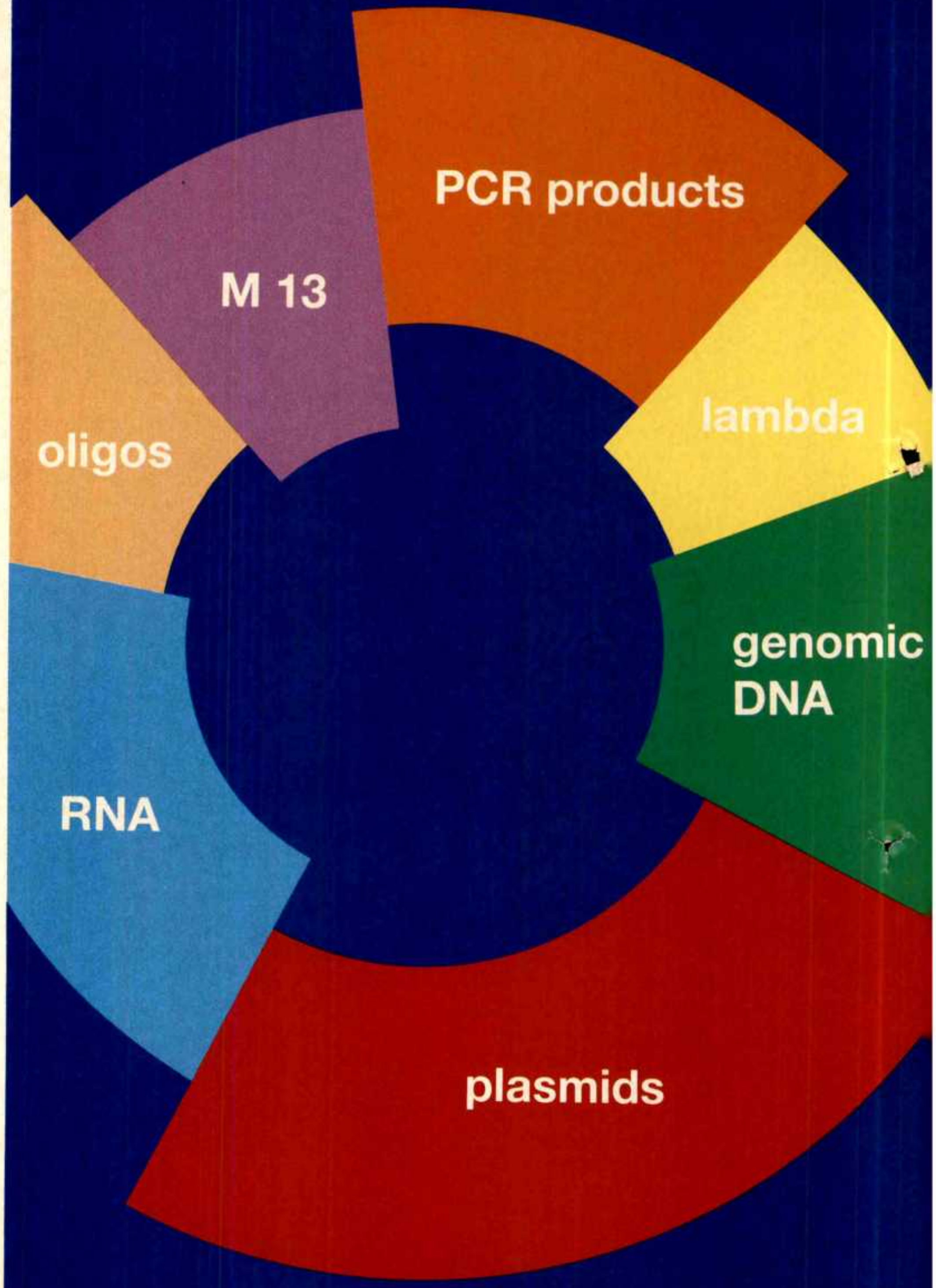

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Pulsar seen in SN1987A remnant?

Optical pulsations may have been detected from the region of the Magellanic Cloud supernova SN1987A by astronomers in Brazil. If confirmed, the observations probably presage pulsed X-ray emission.

THE announcement on 3 October, on astronomers' worldwide electronic mail networks, of the discovery of 18·4 millisecond optical pulsations in the Magellanic Cloud supernova, SN1987A, may be one of the missing pieces in a jigsaw not yet complete. The announcement, by astronomers in São Paulo, Brazil, came just after a meeting on Elba on the supernova* devoted in part to explaining why pulsations had not yet been seen. Astronomers from the European Southern Observatory had shown a long list of upper limits, demonstrating the fruitlessness thus far of their search for optical pulsations. The Brazilian detection shows that luck, too, is an essential ingredient of discovery.

The Brazilian astronomers, F. Jablonski, R. Baptista, C. D. Gneiding, J. E. Steiner and F. Elizalde, claim the detection with the 1·6 metre telescope of the Laboratorio Nacional de Astrofisica in southeast Brazil, of quasiperiodic oscillations with centroid frequencies near 54·5 Hz (a period of 18·3 milliseconds) and bandwidth 0·05 Hz: the oscillations were first detected on 17 September, the day on which the Elba conference began, and again on 28 September.

The Brazilian group interprets the 18·3-millisecond optical signal as reprocessing of the beam from the rotating neutron star formed in SN1987A. The pulsations would be at a well-defined frequency if the Earth were in the sweep of the emission beam of the pulsar as it rotates, because the rotation of a neutron star is very stable. The pulsations seen in Brazil were not of this nature; their frequency changed noticeably from hour to hour.

That is why the Brazilian astronomers propose that the neutron star is oriented pole-on towards the Earth so that we see the flashes not directly but indirectly, reflected from something nearby — infalling or orbiting clouds, or, less plausibly, a companion star. The result is therefore a train of pulses Doppler-shifted by reflection in moving mirrors.

These observations remain to be confirmed. (Previously reported 2 kHz pulsations are now known to be due to inductive pick-up in the equipment but, while 54 Hz is curiously near mains frequency, the Brazilians list an impressive set of null observations of stars other than the supernova.) But confirmation will provide the second piece of evidence, and the long-awaited confirmation, that SN1987A produced a neutron star when it exploded on 23 February 1987. The first evidence was, of course, the neutrino emission then seen by the Kamiokande neutrino detector in Gifu, Japan, and its sister detector, known as IMB, in Ohio. Since then no further trace of the neutron star had until now been seen.

The total ultraviolet, optical and infrared emission from the supernova is about 500 times more than the pulsed emission and arises from reprocessed energy from the radioactive decay of cobalt-56, itself produced from radioactive nickel-56 formed in the explosion. The light curve of the supernova (the evolution of its brightness) is still, after three years, accurately tracking the exponential decay of radioactive cobalt, uncontaminated by evidence even of longer-lived isotopes such as cobalt-57 and titanium-44, expected to have been formed with nickel-56.

The most serious persisting doubt about the existence of a neutron star in SN1987A is the lack, for two years from August 1987, of the X-rays expected by degradation of the γ-rays emitted by cobalt-56. SN1987A has now faded from the view of all active X-ray telescopes. It is not now seen during the monthly inspections by the Japanese GINGA satellite and was not seen in the brief glance on 23 May this year by the Soviet GRANAT instrument. This last of these limits was at a level of just a few thousandths of the luminosity of the Crab Nebula, itself powered by an ageing 950-year-old pulsar which would be expected to be much less powerful than a lusty three-year-old.

The strongest upper limit on the X-ray luminosity is from a test observation of the Magellanic Cloud during the so-called performance verification phase of the West German X-ray telescope on the ROSAT satellite. The supernova was not seen above a luminosity of 5×10^{34} erg s^{-1}.

X-ray emission from a neutron star is expected when material falls on to it and releases gravitational potential energy. The material can be drawn in onto a newly formed neutron star: it can be driven back from the expanding ejecta by a reverse shock in the outflowing material and sucked in (according to a suggestion originally made by Stirling Colgate, Los Alamos National Laboratory) by the cooling of the neutron star's surface by continuing neutrino emission. But, however much material there is (within limits), the luminosity of the radiation is pushed up to 3×10^{38} erg s^{-1} by a self-limiting process — if too much material falls in, so that the luminosity rises above this limit, radiation pressure expels the excess.

The infalling material, if there is any, quenches pulsations from the neutron star, so that the gravitational potential energy may at first show up as steady optical emission from the kind of atmosphere that forms on an isolated neutron star (R. Chevalier, University of Virginia). The atmosphere is unstable and dissipates on a timescale of about a year, ultimately revealing emission from the neutron star itself. Perhaps that is why the optical pulsations have only now become visible.

The present lack of unpulsed X-ray emission shows that the region around the neutron star is now very clean, with no material there to fall back. That is borne out by the discovery in August that the supernova has turned on again in radio emission (it was first seen at radio frequencies soon after the explosion in 1987, but the radio power quickly died away). Australian radioastronomers at the Molongolo Observatory Synthesis Telescope (immodestly known as the MOST) have recently seen the supernova progressively increasing in brightness at levels upwards of 1 millijansky. At the Elba conference, it was calculated that the radio flux was then increasing as t^{69}, measured since the 1987 explosion — a step-function switch-on like a window opening.

There was speculation at Elba about the explanation of this phenomenon. Either the pulsar is revealing itself or some of the expanding ejected body of the supernova has hit a lump of circumstellar material. If the former, the appearance of the radio emission would be evidence that the line of sight has cleared of material, letting the radio emission out. Intercontinental aperture synthesis techniques could distinguish the two possibilities; if the radio signal were to coincide with the supernova, then that is probably the pulsar; if the radio source is displaced by a fraction of an arcsecond, then it probably comes from the ejecta instead. But what is needed first of all is confirmation of the Brazilian result. Then, if there really is pulsed optical emission, the detection of pulsed X-ray emission is likely soon to follow.

Paul Murdin

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*Workshop on SN1987A and other supernovae, 17-22 September, Marciana Marina, Elba.

EVOLUTION

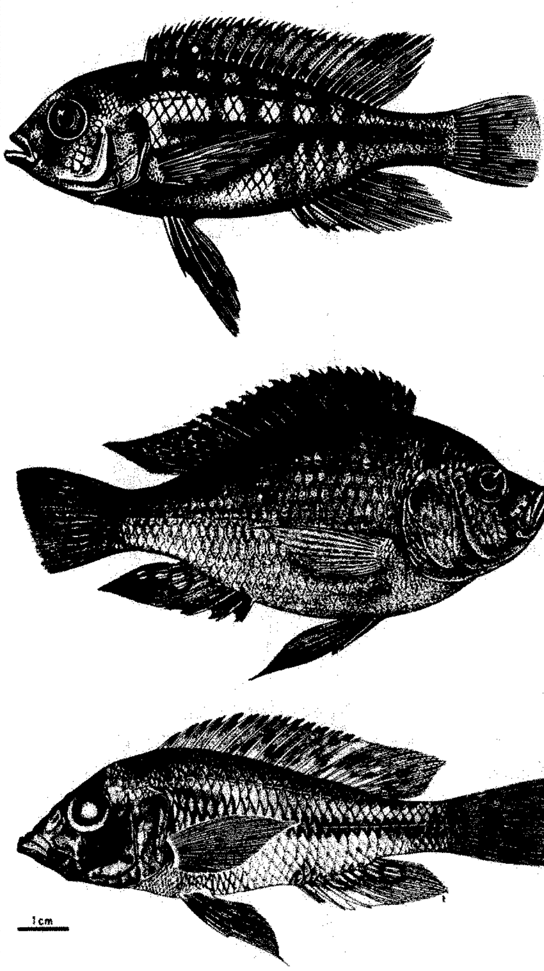
Flocks of African fishes

John C. Avise

MORPHOLOGICAL and molecular evolution can march to the beats of different drummers. Thus horseshoe crabs, 'living fossils' that have changed little in morphology over tens of millions of years, exhibit 'normal' patterns of genetic variation and divergence in proteins and DNA[1,2], whereas humans and chimps, which differ so obviously in anatomy and way of life, are more than 99 per cent identical in polypeptide sequences[3]. Even against such examples, the apparent decoupling of morphological and molecular evolution reported for African rift-valley lake fishes on page 550 of this issue is stunning. Meyer *et al.*[4] report that 14 assayed species, representing nine genera of cichlids endemic to Lake Victoria, show almost no differentiation at some 800 nucleotide positions in the cytochrome *b* gene, two transfer RNA genes and the normally highly variable portion of the control region of mitochondrial (mt) DNA. The mean level of mtDNA sequence divergence among these species and genera is less than that within a single species of horseshoe crab[2], or within the human species, which itself exhibits low intraspecific mtDNA differentiation compared to many vertebrates, including other fishes.

The cichlid species flocks in Lake Victoria and other African rift lakes have long intrigued biologists as prime examples of 'explosive evolution'[5]. Lake Victoria is less than one million years old, yet it contains some 200 species of cichlids, almost all of which are endemic. This assemblage exhibits moderate diversity in external morphology (see figure), but far more striking are the ecological and trophic specializations represented[6,7]. There are algae grazers, plankton and detritus feeders, pharyngeal snail crushers, and insect and fish predators. Some species are paedophages, eating fish embryos by engulfing the snout of a mouth-brooding female and forcing her to jettison the brood. One species feeds on scales rasped from tail fins, while another (in Lake Malawi) plucks the eyes from other fishes. The discovery of a near lack of mtDNA differentiation among representative Lake Victoria cichlids implies that this remarkable kind of organismal diversity evolved very recently (within the past 200,000 years), probably from a single common ancestor within the lake.

The hypothesis of recent monophyly for the Lake Victoria flock is further supported by mtDNA comparisons involving other African cichlids[4]. For example, assayed species in nearby Lake Malawi differed from those in Lake Victoria by more than 50 base substitutions, whereas the Lake Victoria cichlids differed from one another by an average of only three mtDNA mutations. Among 20 members surveyed of the 200 or so endemic cichlids in Lake Malawi, two distinct mtDNA lineages were observed, but these remained much more closely related to one another than to any non-Malawi cichlids examined. Thus the molecular findings appear to eliminate an alternative picture for the Lake Victoria and Lake Malawi species flocks — that they might represent the polyphyletic products of numerous invasions by taxa that had become highly differentiated before each lake's formation.



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Examples of cichlid species from Lake Victoria assayed by Meyer *et al.*[4]. Top, *Ptyochromis sauvagei*; middle, *Lipochromis obesus*; bottom, *Astatotilapia piceatus*. (Reproduced from ref. 7.)

The phylogeny and taxonomy of the African cichlid flocks have long been under dispute. Traditionally, most of the described species (over 500 of them) were placed in a single genus, *Haplochromis*, but in recent years a morphological reanalysis based on hennigian principles prompted a taxonomic splitting into a large number of genera and subgenera[7]. Many of these taxa have representatives in at least four of the African great lakes, such that "the overall picture is one of a super-flock comprised of several lineages whose members cut across the boundaries imposed by the present-day lake shores"[8]. The molecular findings of Meyer *et al.*[4] stand in stark opposition to this concept. If the mtDNA results are corroborated and extended to many additional haplochromine species, yet another taxonomic realignment will be in order, one in which the lineages recognized would coincide almost perfectly with the present-day lake-shore boundaries.

The phylogenetic findings with mtDNA set the stage for further research on the ecology and genetics of African great-lake fishes. Did the intra-lacustrine speciations take place sympatrically, or did they occur at times of geomorphological subdivision of a lake basin? Is there a simple genetic basis to the ecological and morphological shifts, and are particular morpho-genes involved? Could at least some of the morphological shifts represent phenotypic or ontogenetic plasticity within species, as has been suggested for trophic polymorphisms in some New World cichlid fishes[9]? (Additional assays of nuclear genes are needed to decide whether the gene pools of all morpho-species are truly distinct.) Is there some peculiarity in the genetic makeup, behaviour or ecology of cichlid fishes that predisposes them to explosive evolutionary radiation, whereas other fish taxa in these lakes appear to evolve at normal rates? What are the ecological and behavioural ramifications of habitat partitioning by so many closely related forms? These are all obvious but difficult questions. The findings of Meyer *et al.*[4] at least show that there really is a phen-

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omenal pattern of within-lake evolutionary radiation to be understood.

The cichlids of the great African lakes are the most spectacular of a small number of fish species flocks in drainage basins scattered around the world[10]. Most are threatened with extinction from anthropogenic causes, and indeed the cyprinid flock in the Philippines' Lake Lanao has already been lost. The African cichlids are also in rapid decline, primarily because of the introduction of exotic predatory fish. The great African rift lakes and their faunas will someday disappear as tectonic plate movements continue to split the continent. Premature closure of this extraordinary evolutionary theatre and play through human activities would be a biological tragedy of the first order. ☐

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ASTROPHYSICS

X-ray burster is theory buster

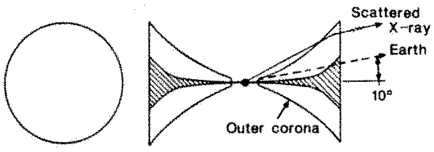
Michael Garcia

THE hallways at the most recent meeting of X-ray astronomers (held in Bologna in September) were buzzing with rumours about the latest results from Japan's GINGA X-ray astronomy satellite. One of the more spectacular rumours was that the satellite had detected an X-ray burst from the bright X-ray source (AC211) in the centre of the globular cluster M15. Not only was this the first detection of an X-ray burst from this object, but the rumour was that it was a truly spectacular burst — unusually bright and long, and containing features rarely (if ever) seen in X-ray bursts. On page 534 of this issue[1], Dotani *et al.* reveal that the burst was every bit as spectacular as rumoured.

X-ray bursts are commonly seen from low-mass X-ray binaries (LMXBs), which are short-period binaries (orbital periods of a week or less) containing a neutron star or black hole which accretes matter from a companion star. The bursts occur when sufficient matter has accreted on to the neutron star to fuel a runaway thermonuclear reaction. Once started, the entire surface of the neutron star ignites within about a second, and it burns for a few hundred seconds. LMXBs are seen throughout our Galaxy, but are concentrated in globular clusters which have stellar densities sufficient to allow the formation of very close binaries through near collisions between stars.

AC211 is unique among the globular-cluster LMXBs because it is the only one for which an optical counterpart has been found. Optical studies have allowed astronomers to determine its structure to greater precision than any other cluster LMXB. For example, we know the orbital period is 8.5 hours, and that the optical light is strongly modulated at this period[2]. The heliocentric radial velocity of the system is also modulated at this period, but the mean velocity is offset from the host cluster's by about 150 km s^{-1}. This remarkable fact was initially interpreted[3] to mean that the binary was being thrown out of the globular cluster by the same stellar collisions as formed it. Subsequent studies have shown that the lines with these high radial velocities are formed in a wind which flows out from the system, and that AC211 itself is not being ejected from the cluster[4].

The strong optical modulation suggests that the binary system is viewed nearly edge-on, as this geometry enhances the observed modulation caused by two stars orbiting each other. The ratio of the X-ray to optical luminosity (L_X/L_{opt}) is much lower than typical[3] and has also been taken as evidence that the system is viewed nearly edge on, and that the accretion disk blocks our view of the central neutron star, therefore reducing the apparent X-ray luminosity. The X-rays we do see are presumed to be scattered off an accretion-disk corona (ADC) surrounding the central source, and extending above the accretion disk[5] (see figure). There are at least five other LMXBs that show evidence of such a corona[6], so before the announcement of the GINGA burst this model of AC211 was reasonably well accepted.



This schematic diagram of an accretion-disk corona source shows how the accretion disk (shaded) can block our view of the central neutron star, therefore lowering the apparent X-ray luminosity of the LMXB. The dense neutron star is on the right and its companion, on the left. (From ref. 5.)

However, the ADC model implies that even during an X-ray burst we should see only a fraction of the total X-ray emission, and this indeed what was seen[7] during a burst from another ADC source, 4U2129+47. At the peak of the burst from AC211 the X-ray flux reached about twice the Eddington limit — the theoretical limit which the flux cannot exceed. The violation of this limit is in itself disturbing, but may be at least partially explained if the matter being accreted in AC211 is primordial and therefore devoid of any elements heavier than helium (atomic absorption contributes to the limit). But the idea that we are only seeing a fraction of the total X-rays when we see twice the theoretical limit is clearly untenable, so that the idea that the accretion disk blocks our view of the central neutron star must be wrong. The only way to salvage it is to assume that the disk changes shape during the burst, or that the burst was from another (previously unknown) source within a few arcseconds of AC211.

Rather than invoke such *ad hoc* explanations, it seems more reasonable to attribute the low ratio L_X/L_{opt} to an unusually high optical luminosity, as suggested by Fabian *et al.*[8]. This idea seemed an unnecessary complication when first put forward, but with the discovery of this bright burst it now seems to have been remarkably prescient. The known lack of heavy elements in the globular cluster M15 (and presumably in AC211 as well) is conducive to forming an optically bright accretion-disk corona. Fabian *et al.* point out that the physical conditions which allow an optically bright accretion-disk corona would also allow a rather large outflow of material off the surface of the disk. This material may be exactly that seen leaving the system at a few hundred kilometres per second[4,9]. The rate of mass loss in this wind is high enough to cause accelerated and unstable evolution of the binary system.

This evolution could ultimately cause the orbital period of AC211 to be drastically decreased. Interestingly, the only other cluster LMXB for which the orbital period is known is 4U1820−30, in the cluter NGC6624, which has the shortest orbital period of any known binary — 11 minutes (ref. 10). Bailyn and Grindlay[11] have suggested that AC211 will evolve into a system like 4U1820−30; the rapid and unstable evolution implied by the idea proposed by Fabian *et al.* seems to support this suggestion. ☐

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J. S. Bell (1928–1990)

John Stewart Bell, who died unexpectedly on 1 October, became famous for his work on quantum mechanics, but he also made many major contributions to accelerator physics — many-body theory — especially as applied to nuclei–quantum field theory and particle physics. His successful prediction that neutrinos would suffer nuclear 'shadowing', despite having a mean free path in matter of millions of miles, is just one example of his many 'conventional' contributions.

His work on the 'Alder–Bell–Jackiw' anomaly, which showed that certain symmetries of classical theories cannot be maintained in quantum theories, led not only to important constraints on models of elementary particles, but also to some very surprising and deep connections between physics and geometry which are still being explored.

Bell's first contribution to what he called "the problem of quantum mechanics" was to demolish von Neumann's celebrated theorem that purported to show that "quantum mechanics would have to be objectively false in order that another description of the elementary process than the statistical one be possible". He then showed that certain predictions of quantum mechanics cannot be reproduced by any 'local' theory in which the results of a measurement on one system are unaffected by operations on a distant system with which it interacted in the past. Although "only to be expected", in Bell's phrase, the subsequent verification of these predictions was of fundamental importance.

His masterly expositions of the shaky state of the foundations of quantum mechanics, in which he stressed particularly that in the absence of a definition of what constitutes a 'measurement' — or 'experiment' as he preferred — the predictions are in principle ambiguous, did much to rock the "complacent" views of most physicists. His own work is the most important contribution to 'quantum philosophy' since the birth of quantum mechanics, although his clarifications served only to deepen the fundamental mysteries of the subject.

Total honesty and decency characterized all John's acts. Although he regarded as "good fun" the contacts with the Dalai Lama, the Maharishi, and assorted mystics that resulted from the notoriety of his work on quantum mechanics, he was a private person of great modesty. His many friends will sorely miss his quiet, red-headed and bearded figure padding along the corridors of CERN in sandals, his gentle humour, his delightful use of the English language delivered in soft Ulster tones, his advice, and of course his views of Physics.

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TRANSCRIPTIONAL CONTROL

Scissors and helical forks

Robert T. Sauer

THE recognition of DNA by gene-regulatory proteins is often mediated by simple structural motifs that are part of a single protein domain. One such domain is found in the highly conserved 'leucine-zipper' family of eukaryotic transcription factors, which includes C/EBP, Fos, Jun and GCN4. The DNA-binding domains of these proteins contain adjacent basic and leucine-zipper regions, each of about 30–40 amino acids. The leucine-zipper region mediates dimer formation via a coiled-coil arrangement of parallel α helices, in which leucines occur every seven residues[1,2]. The preceding sequence, which is rich in basic residues, has been proposed to make direct contact with DNA, as binding requires both the basic region and the leucine-zipper region, and mutations in either sequence can prevent binding. Recent studies[3-7], including those reported by Weiss *et al.*[6] and Patel *et al.*[7] on pages 575 and 572 of this issue, now provide evidence which suggests that the basic regions of leucine-zipper proteins assume a stable α-helical structure only when bound to a specific DNA site.

Earlier this year, Talanian *et al.*[3] showed that the basic region contains all of the information necessary for DNA recognition. Using a synthetic peptide containing the basic region of GCN4 followed by a Gly-Gly-Cys linker, they observed strong, sequence-specific DNA binding, as long as the peptide dimer was linked by a disulphide bond and the binding experiments were performed at low temperature. Under these conditions, the normal dimerization function of the leucine-zipper region can be replaced by a disulphide crosslink.

The basic region of GCN4, as an isolated peptide or attached to the leucine-zipper region, is about 25 per cent α-helical in solution, as judged by circular dichroism[3,5,6]. This indicates either that this region contains a short region of stable helix or, more likely, that it consists of an ensemble of partially folded forms[6]. On binding to a specific DNA site, however, the basic region becomes almost completely α-helical[3,5,6]. So binding of GCN4 peptides to DNA must be accompanied by a significant change in the conformation of the basic region. The conformations of the basic regions of C/EPB, Jun and Jun–Fos also seem to be altered on binding to DNA[4,7], presumably as a consequence of a similar coil-to-helix transition. One might worry that the conformational change accompanying DNA binding in all of these systems is an artefact, occurring in the peptide fragments used for these studies but not in the intact leucine-zipper proteins from which they derive. But peptide fragments from proteins such as GCN4 seem to have specific DNA-binding affinities comparable to those of the intact proteins[6]; this would not be expected if the DNA-binding surface were properly structured in one molecule but not the other. Also, intact GCN4 is reported to show an increase in its helix content, similar to that of the peptides, when it binds to specific DNA[5].

The α-helical nature of the DNA-bound basic region had been independently predicted by two groups[5,8], providing impetus for some of the circular dichroism experiments described above. These predictions were based on the absence of helix-breaking residues such as proline and glycine in the basic region, the periodic distribution of conserved residues in this region, and the invariant spacing between the basic and zipper regions. Moreover, both groups proposed that the DNA-bound protein would have a Y-shaped conformation, with the dimeric leucine zipper forming the base of the Y and the helical basic regions diverging to form the arms.

In the 'scissors grip' model of Vinson *et al.*[8], the two basic regions approach the DNA at a common point near the cleft of the Y, and then track in opposite directions along the major grooves of the dyad symmetric site. Interactions with the major groove are required to account for the known positions of protein–DNA contact[8-10]. A kink or sharp bend in each α helix allows these regions to follow the curve of the major groove, thereby wrapping around the DNA. The 'induced helical fork' model of O'Neil *et al.*[5] is fundamentally similar to the scissors grip model, although the α helices in this model are not kinked and a specific set of DNA contact residues is proposed. Although the details of these models need to be tested, their basic features are likely to be correct. It therefore seems that the leucine-zipper proteins use a new DNA-binding motif. The scissors grip and helical fork models are also attractive because they provide a rationale for the observed change in conformation that accompanies DNA binding. In solution, the helical arms of the Y would not be stabilized by tertiary interactions, and hence the corresponding basic regions would be expected to be relatively unstructured. In the complex, stabilizing tertiary contacts would be provided by the interactions of the α helices with the DNA.

The conformational transition of [illegible] basic region may be an important or even essential feature of the activities of the leucine-zipper proteins. From a thermodynamic point of view, the disorder–order transition that accompanies DNA binding could provide a mechanism for limiting the free energy of binding, while still allowing maximal specificity of binding. Although the binding energy could also be reduced by interfering with contacts in the complex, this would lead to a reduction in specificity. The basic region might have to be loosely structured in solution for kinetic reasons. In the scissors grip model, the protein forms a type of molecular clamp by encircling the DNA[8], and large kinetic barriers in the assembly and disassembly of the complex would be expected unless the basic regions were flexible[4,6]. Weiss *et al.*[6] point out that a similar situation occurs in the λ repressor, where the N-terminal arms of the protein, which wrap around the DNA in the complex[11], are flexible in solution.

Another potential role for the disorder–order transition is suggested by O'Neil *et al.*[5]. They found that the basic region has an intermediate level of helix when bound to nonspecific DNA and speculate that this may allow the protein to perform an efficient 'search' of the DNA for the specific recognition site. Finally, Patel *et al.*[7] reason that the conformation of the activation domains of Fos and Jun, which are close to the basic region, may also be altered in the absence of DNA. This, they argue, might provide a way in which regulatory molecules that are not bound to DNA could avoid 'squelching' or competing for components that are needed by the transcriptional machinery.

Conformational transitions are not limited to the basic region of the leucine-zipper proteins. Peptides containing the basic and leucine-zipper regions of GCN4 undergo a concentration-dependent transition in the micromolar range, reflecting a concerted dissociation and unfolding of the coiled-coil structure of the leucine-zipper region[6]. So the peptide is predominantly monomeric and unfolded at the nanomolar concentrations at which specific DNA binding occurs *in vitro*. This should result in a cooperative binding reaction, with DNA binding showing a second-order dependence on the concentration of free protein. Does cooperativity of this type operate in the cell? The answer depends on whether the intact protein is predominantly monomeric or dimeric *in vivo*. Because the intracellular concentrations of most leucine-zipper proteins are low, most free proteins would be expected to be monomeric if the behaviour of the peptide is a good model for the intact protein, although binding to non-specific DNA could stabilize the dimer. If the DNA-binding domains of leucine-zipper proteins are actually unfolded in the cell, then one may also wonder whether these regions are complexed with molecular chaperonins, and, if so, whether such interactions have any regulatory role.

Understanding the stability of the paired α helices of the leucine-zipper region is crucial for determining the rules that govern the formation of particular homodimers such as Jun–Jun or heterodimers such as Jun–Fos. But it is still not clear which sequence elements provide the specificity for these interactions, and, indeed, what determines whether these coiled-coil interactions will be parallel or antiparallel. Until recently, most structural information on parallel coiled-coils came from model building or low-resolution diffraction studies on proteins such as tropomyosin. However, higher resolution detail should soon be available from the recently completed crystal structure of the parallel coiled-coil region of GCN4 (E.K. O'Shea *et al.*, personal communication). A high-resolution view of an antiparallel α-helical coiled-coil, roughly 60 Å in length, can be found in the crystal structure of seryl-tRNA synthetase, which was reported last month[12]. This structure is stabilized by a 'ladder' of Leu–Leu and Leu–Ile interactions and a variety of inter-helical and intra-helical salt-bridges and hydrogen bonds. Comparison of the structures of these parallel and antiparallel α helices should be helpful in untangling the mysteries of coiled-coils. ☐

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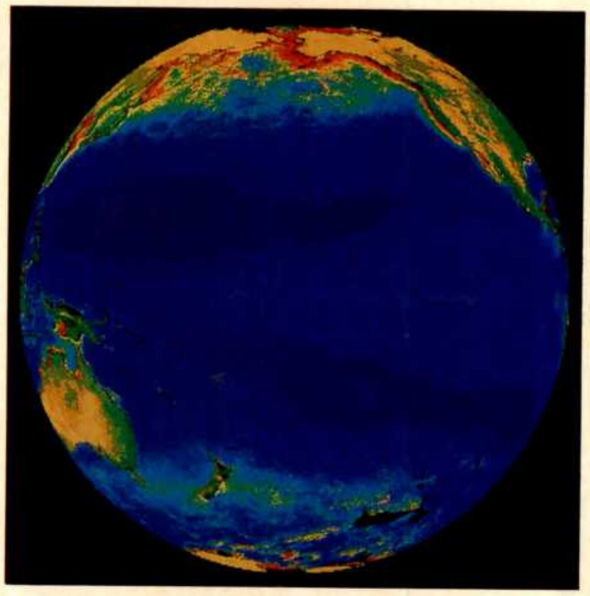
Solar power to the plankton



IT IS tempting to think of the Pacific Ocean as a vast watery waste that separates Asia and Australasia from the Americas, with little direct effect on the bordering continents. Yet the two-yearly climate cycles associated with the El Niño events bear powerful witness to the importance of the ocean. El Niño events, which can bring drought to Australasia, and excessive rainfall to Peru, are closely tied to cyclical variations in the surface temperature of the Pacific Ocean. Attempts to understand the mechanism relating the two cycles have been hindered by a disparity between the estimated flux of heat across the ocean–atmosphere interface and that calculated in computer models. Marlon R. Lewis and colleagues, who describe their work on page 543 of this issue, have used images from the NIMBUS-7 satellite to estimate the transparency of the sea surface to solar radiation. The Coastal Zone Color Scanner, an experimental instrument included in the satellite only for testing, proved so successful that its planned one-year life was extended to seven. The image above is a compilation of the full seven-year's data: Lewis and colleagues used just the first year's, which are the most reliable. The image is coded purple, for the most transparent water, through to green and yellow, for the most turbid regions. Penetration of solar radiation through the ocean's most transparent regions means that less of its heat is deposited near the ocean–atmosphere interface than had been supposed. This accounts neatly for the discrepancy between model and estimated sea surface temperatures. Phytoplankton, the main cause of the ocean surface's opacity, are also the principal source, through changes in their abundance, of interannual variations in the atmospheric concentration of carbon dioxide (see U. Siegenthaler's News and Views article, *Nature* 345, 295; 1990). The new work by Lewis and colleagues further underlines the importance of these apparently benign lifeforms in climatic feedback cycles.

R.P.

GENERAL RELATIVITY

Space-based gravity tests

Clifford M. Will

WHAT does the future hold, if anything, for experimental studies of gravitation? This was the central question at a recent conference* held to honour the memory of William M. Fairbank, one of the most enthusiastic and forceful advocates for experimental gravitation, who died last year[1]. Noted for several discoveries in low-temperature physics, Fairbank turned his attention during the 1960s to the application of cryogenic techniques to precision gravity experiments, and his Stanford laboratory soon became a centre for such work. This included the construction of bar detectors for gravitational waves, attempts to measure the free fall of positrons, and an experiment to measure the einsteinian 'dragging of inertial frames' by the rotating Earth using an orbiting gyroscope.

Coincidentally, the conference also came within two months of the 75th anniversary of Einstein's final formulation of the general theory of relativity. Based on the principle of equivalence, or the equality of free-fall acceleration of different bodies, general relativity accounts for gravity as a consequence of curved space-time. Although the theory was pretty much in hibernation for its first 45 years, the past 30 have witnessed a renaissance of the subject, not least in the area of experimental gravitation. In fact 1960–80 was something of a golden era for testing relativity, during which most of the theory's predictions for effects in the Solar System (light deflection, gravitational redshift, retardation of light, the advance of Mercury's perihelion and the equivalence principle for the inertial and gravitational masses of the Earth and Moon) were verified to accuracies ranging from 1 to 0.01 per cent. And observations of the decaying orbit of the remarkable binary pulsar confirmed the theory's predictions for gravitational-wave damping[2,3].

For all the painstaking and time-consuming work that went into the experimental tests of the golden era, they were somehow easy, for they were done at a time when advances in technology (atomic clocks, radio and radar astronomy, space exploration) met the demands for checking the validity of general relativity, which arose from astronomical discoveries. The necessary experiments *could* be done with good precision, and so they were.

SPACE-BASED TESTS FOR GRAVITY

Experiment	Quantity measured	Accuracy proposed
Satellite test of equivalence principle	Differential acceleration of different materials	10^{-17}
Optical interferometer in space	Light deflection	10^{-6}
Laser-ranged Earth satellite	Frame-dragging of orbit plane	2×10^{-3} arcsec yr^{-1}
Atomic clock on Solar Probe	Gravitational redshift	10^{-10}
Microwave interferometer in space	Gravity-wave strain at 10^{-2} Hz	10^{-18}
Laser interferometer in space	Gravity-wave strain at 10^{-4} Hz	10^{-21}
Gravity gradiometer in space	Curvature tensor	10^{-19} g cm^{-1}

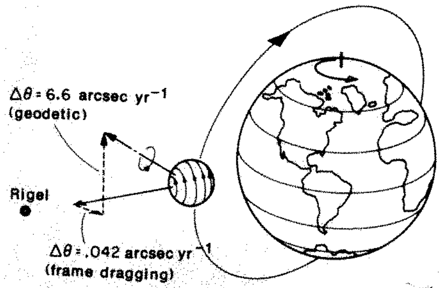
In contrast, the experiments envisaged for the future require almost heroic advances in technology and precision techniques to reach the required sensitivities. *Near Zero*, a *Festschrift* for Fairbank[4] published in 1988, neatly characterizes the requirements for many of these experiments: near-zero temperature (10^{-3} K noise temperature in gravity-wave antennae); near-zero magnetic field (10^{-7} gauss in the gyroscope experiment); and near-zero acceleration (10^{-11} g for a satellite test of the equivalence principle). Why? Because the effects to be measured are also near zero.

Nothing illustrates this better than the gyroscope experiment, denoted Gravity Probe B (GP-B) by NASA, which was described in detail at the meeting (C.W.F. Everitt, Stanford University). The plan is to place a set of four superconducting-niobium-coated, quartz gyroscopes into polar Earth orbit to measure the predicted 42 milliarcsecond per year precession of the spin axes to better than 0.5 per cent.

The frame-dragging effect responsible for this precession is a rotation of the axes of local inertial frames relative to distant stars caused by the rotation of the Earth (see figure). The effect is sometimes called gravito-magnetism because it is analogous to the precession of a magnetic moment outside a rotating charged body. Apart from being an important testable prediction of general relativity, it plays a central role in supermassive-black-hole models for the formation of jets in quasars, and the notion that distant matter can induce rotation of local inertial frames has obvious machian overtones. Thus the direct detection of this effect is a goal worth reaching, despite the difficulties. The required precision implies measuring a precession rate which is another near zero: 10^{-17} radians per second.

In the 1960s and 1970s, what was needed to achieve this seemed beyond the pale: the 4-cm-diameter gyroscopes would have to be homogeneous and spherical to a few parts in 10^7; the spacecraft carrying the experiment would have to be free of drag (residual accelerations less than 10^{-12} g); an onboard telescope would be needed, capable of providing a reference direction stable to 0.1 milliarcseconds per year; and so on. But recently the GP-B laboratory successfully conducted an Earth-bound test of the integrated gyroscope–cryogenics–telescope system (M. Tater, Stanford University), showing that many of the requirements have now been met and that others will follow once the device reaches the low-gravity environment of space. The current plan calls for an orbiting mission around 1996.

But GP-B is not the only space-based gravitation experiment being planned. Others for the next decade are at various stages of development: some are just conceptual; for others, the hardware is being developed. Their goals include detection of low-frequency gravitational waves (seismic noise restricts Earth-bound detectors to seeking frequencies above 1 hertz), orders-of-magnitude improvement of current tests of general relativity, and probes of higher-order relativistic gravitational effects. Some of the projects


$\Delta\theta$ = 6.6 arcsec yr^{-1}
(geodetic)
Rigel
$\Delta\theta$ = .042 arcsec yr^{-1}
(frame dragging)

Precession effects due to relativity in a gyroscope experiment orbiting the Earth. Detecting the 42 milliarcsecond change each year due to 'frame dragging' will require remarkable, but achievable precision. (From C.W.F. Everitt in ref. 4, page 589.)

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* *Relativistic Gravitational Experiments in Space*, Rome, 10–14 September 1990.

discussed at the meeting are outlined in the table.

If these experiments are so difficult and take so long, and require access to space (which has lately become such a precious commodity), why bother to do them? The answer, in the case of gravity-wave detectors, is that a new astronomical window will open up, revealing information about violent and dynamic cosmic events. Furthermore, only space-based detectors can 'see' the known sources of gravity waves, such as close binary systems.

As for the basic notion of gravitational tests, general relativity is a fundamental theory of nature, and should be given the best empirical backing we can provide. More importantly, its predictions are fixed; the theory contains nothing that can be adjusted to accommodate any experimental deviations. Thus any test of general relativity is potentially a deadly test, for any verified deviations would kill the theory. As his quest for fractionally charged free quarks showed, Fairbank loved to try experiments that might overthrow conventional wisdom; one suspects that even an outside chance of demolishing general relativity would have kept him in the game for many years to come. ☐

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PLANT PATHOLOGY

The race for resistance genes

Geoffrey North

PLANTS have defence mechanisms by which they can resist infection by potentially pathogenic microorganisms. Like the immune systems of higher animals, these mechanisms work by first recognizing foreign molecules produced by the invading pathogen. The recognition repertoire of plants, however, lacks the developmental flexibility of animal immune systems, and is set for the life of a particular plant by the collection of so-called 'resistance' genes it happens to inherit. The cloning of resistance genes is an important goal in plant pathology, and on the evidence of a recent meeting* it should not be long before it is achieved.

Resistance genes commonly exhibit what are known as 'gene-for-gene' interactions with specific genes carried by the pathogen, which are perhaps misleadingly referred to as 'avirulence' genes. Gene-for-gene interactions are recognized by a simple pattern of inheritance such that plants carrying a particular resistance gene can recognize, and are thus resistant to, pathogens carrying the complementary avirulence gene, but if either resistance gene or avirulence gene is absent a virulent infection ensues. The resistance gene is assumed to code for some kind of receptor molecule which signals the presence of the product of its complementary avirulence gene, setting off a 'hypersensitive reaction' by which the plant can isolate the pathogen at the site of infection and prevent its spread throughout the plant.

Avirulence genes are thus named because their presence prevents virulent infections on host plants carrying the appropriate resistance genes. It is presumed that pathogens do not carry avirulence genes to alert host plants to their presence, and work on the pathogen *Xanthomonas campestris*, which causes bacterial spot disease of tomato and *Capsicum*, provides evidence that at least some avirulence genes are required for full virulence of the pathogen on susceptible hosts (Kearny, B. & Staskawicz, B. J. *Nature* **346**, 385–386; 1990).

This property may explain the high degree of conservation of the avirulence gene *avrBs2* among *X. campestris* strains, and why its complementary resistance gene *Bs2* provides stable field resistance to bacterial spot disease in *Capsicum* (B. J. Staskawicz, University of California, Berkeley). The hope is that the *Bs2* gene will work similarly in other crop plants, to which it could be transferred when it has been cloned.

A number of routes are being taken to clone plant resistance genes in the absence of a defined biochemical product. For example, efforts are being made to exploit the advantages of the small crucifer *Arabidopsis thaliana*, currently the favourite subject of the plant molecular geneticist (F. Ausubel, Massachusetts General Hospital, Boston). Certain *Arabidopsis* lines have resistance genes corresponding to avirulence genes carried by particular strains of *Pseudomonas syringae*, and as a first step Ausubel and colleagues are attempting to isolate mutants that have lost resistance. In particular, they would like to obtain plants with deletion mutations in a resistance gene, which would enable them to apply the technique of 'subtractive hybridization'. Here, genomic DNA with the deletion mutation is hybridized to DNA from the wild-type parental plants in the hope that the non-hybridizing DNA, which can be cloned, would be enriched in sequences representing the deletion. The technique has already been used to clone an *Arabidopsis* gene for an enzyme of gibberellin biosynthesis; the problem, however, is the lack of a straightforward general way of inducing deletion mutations. Using a screen with the chemical mutagen ethylmethanesulphonate, an *Arabidopsis* mutant has been obtained that has lost resistance to a particular strain of *X. campestris* (M. J. Daniels, John Innes Institute, Norwich). But cloning the site of mutation is not an easy step.

A subtractive hybridization procedure is also being used to clone tomato resistance genes to the fungal pathogen *Cladosprium fulvum* (M. Dickinson, John Innes Institute). In this case the subtraction is done not with genomic DNA but with complementary DNA copied from the messenger RNA of plants with and without a particular resistance gene. A number of cDNAs have been identified that seem to be specifically expressed in tomato plants carrying resistance gene *Cf2*, though as yet none has been shown to be the product of the *Cf2* gene.

Other groups are using an approach well known to human geneticists — that of mapping resistance genes relative to polymorphic markers, followed by 'chromosome walking' to the resistance gene. One requirement is a dense enough map of polymorphic markers throughout the genome, and a restriction fragment length polymorphism (RFLP) map is being built up for lettuce and used to map the position of resistance genes to the downy mildew fungus *Bremia lactucae* (R. Michelmore, University of California, Davis). A similar approach is being used to map and ultimately clone tomato resistance genes to *Pseudomonas syringae* pv *tomato* (S. Tanksley, Cornell University). Both of the groups concerned reported how the construction of a map of polymorphic markers is being facilitated by the use of a new technique based on the polymerase chain reaction (PCR). This simply involves the cloning by PCR of regions of the genome picked out by random primers: several regions are picked out by any one set of primers, thus increasing the chance of finding a polymorphism relative to a search using a single-copy probe.

The practical benefits of cloning resistance genes are likely to be considerable. Moreover the enterprise will probably provide insight into the poorly understood processes of signal reception and transduction in plants, as well as into the evolution of resistance in the arms-race between plants and their pathogens. ☐

Geoffrey North is Deputy Biology Editor of Nature.

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* The 5th International Symposium on the Molecular Genetics of Plant–Microbe Interactions, Interlaken, Switzerland, 9–14 September 1990.

ELECTRONICS

Making light of polymers

Lewis Rothberg

THE new light-emitting diode (LED) described by Burroughes *et al.* on page 539 of this issue[1] is one of several applications now being found for semiconducting polymers. Such an LED could be used more conveniently than conventional silicon-based ones for making large-area flat panel displays. And the processing method means that the polymer can easily be spun onto its substrate circuitry directly from solution. The low temperature used in processing also means that there is no risk of damage to other components in the circuit.

Many conjugated polymer molecules behave as quasi one-dimensional semiconductors, owing to the delocalization of π-orbital electrons along the organic backbone chain. The dimensionality, composition and morphology of these materials cause them to behave in very different ways from conventional silicon semiconductors. The hope is that the mechanisms responsible for these differences could allow the organic polymers to be used in novel electrical and optical devices. Besides the LED prepared from poly(*p*-phenylene vinylene) (PPV) by Burroughes *et al.*[1], there have been reports of polymers doped to be as conducting as metals[2] and of polyacetylene induced to have giant nonlinear optical susceptibilities[3], necessary for the development of active optical components.

At a more fundamental level, there remains a great deal of mystery about the nature of the charge carriers in these organic semiconductors. The softness of the polymeric lattice leads to strong coupling between the polymer's electrons and vibrations (phonons) such that it is energetically favourable for the local molecular structure to deform around free charges to form lattice-stabilized quasiparticles[4,5]. The spins and effective masses of these quasiparticles can be very different from those of free electrons and holes, and it is the quasiparticles that are thought to be responsible for the conductivity (and photoconductivity) of the conjugated polymers.

In the case of PPV, Burroughes's colleagues[6] have previously shown that a neutral quasiparticle, the polaronic exciton, is responsible for the material's photoluminescence, which is spectrally identical to the recombination luminescence observed in the LED. As this photoluminescence presumably comes from intrachain electron–hole pairs formed by the absorption of a photon, the authors argue that the injected electrons and holes in the LED travel as 'polarons' which, when they encounter an oppositely charged polaron on the same chain, transiently form quasi one-dimensional excitons which decay radiatively. This idea contrasts with the results from the previous optical work[6] which suggest that bipolarons are the most stable quasiparticles in PPV. The resolution to this intriguing difference will no doubt lead to a better understanding of quasiparticle transport in conjugated systems. ☐

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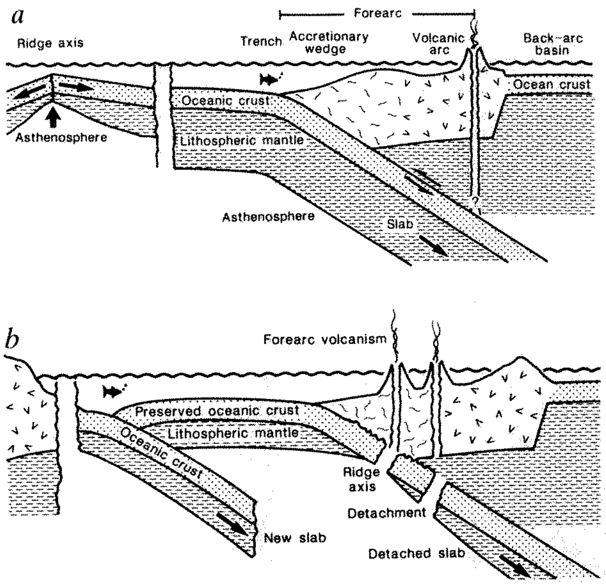
GEOLOGY

A reprieve for ocean crust

Norman H. Sleep

A KEY aspect of plate tectonics is that oceanic crust is formed at mid-oceanic ridge axes, moves horizontally across ocean basins, and is then subducted back into the mantle at trenches. Yet some oceanic crust avoids this fate and is exposed as ophiolites within island arcs or on continents. Ophiolites were described in many localities before their significance was understood. The connection with oceanic crust was made in the late 1960s: rocks dredged from the sea floor were found to be similar to those in ophiolites; the layering of oceanic crust as detected by seismic studies is similar to the observed structure of ophiolites (from marine sediments through crustal rocks of basalt composition down to the mantle); and ophiolites clearly formed in zones of extension similar to those imagined to occur at ridge axes. The processes by which oceanic crust becomes ophiolites, however, have remained poorly understood. In new work, Jilles van der Beukel (*Tectonics* **9**, 825–844; 1990) investigates a mechanism of ophiolite emplacement where a ridge axis is unusually close to a trench and young oceanic crust fails to subduct.



Birth of an ophiolite: *a*, normally the ridge axis is far from the trench. The oceanic crust is carried down with the slab. The sinking of the cold dense slab provides a driving force for the plate motion. Note that the oceanic crust in the backarc may be preserved by virtue of being attached to the crust of the island arc. *b*, The subduction zone on the right dies when the ridge is subducted. The slab detaches preserving the ridge axis and oceanic crust. A new subduction zone begins on the left. The preserved oceanic crust will be uplift when the continent to the left collides with the subduction zone.

As ophiolites on land provide better exposures than could be obtained from any conceivable marine drilling programme, it is essential to know how far geological studies of ophiolites can be extrapolated to oceanic crust or, conversely, whether ophiolite preservation mechanisms create a biased sample. Clearly, ophiolites are unusual in the sense that they escaped subduction. Thus, it is often argued that the crust is also intrinsically different and did not form at a normal mid-ocean ridge. For example, 'autochthonous' ophiolites are formed by spreading in the forearc and backarc basins found on the overriding plate at subduction zones, and along new passive margins, and survive by being attached to buoyant continental crust.

In contrast, there are two-step mechanisms whereby normal oceanic crust is emplaced in the forearc and then uplifted and often exposed as the overriding plate in an arc–continent collision. For example, plate reorganizations that involve formation of a new subduction zone within oceanic crust, either within a plate or by the change of an oceanic transform fault from a strike-slip to a convergent boundary, will by their kinematics leave oceanic crust in the forearc.

van der Beukel's mechanism — failed subduction of young oceanic crust (formed at mid-ocean ridges) — is such a two-step mechanism. However, it is not a geometrical requirement of the gross motions of plates. In part, the mechanism is an inference from geometrical plate tectonics (see figure): mid-ocean ridges spread more or less symmetrically, whereas subduction is one sided. Thus, ridge axes gradually approach trenches so that progressively younger crust enters the trench. If the component of the full spreading rate in the direction of subduction is less than half the subduction rate, the ridge axis collides with the trench and somewhat slower subduction of the plate on the other side of the ridge axis begins. van der Beukel envisages that around the time of the collision, the subducting plate fails mechanically and the slab becomes detached. Subduction restarts on the other side of the ridge axis, preserving a block of the crust in the forearc. The preserved oceanic crust may be normal or it may show chemical effects of its encounter with the arc.

The position of slab detachment and the position where subduction restarts are consequences of the thermal structure and stresses within the subducting plate. (It is geometrically possible for the ridge axis to be peaceably subducted so that no ophiolite is created.) van der Beukel calculates the thermal structure of the subducting plate and the overlying arc and then evaluates what causes the failure of the plate with a flexural model. The main prediction is the position where detachment occurs at depth and thus the geometry of the preserved piece of oceanic crust. For a variety of spreading rates and rheological laws of the plate and the fault zone, detachment occurs at depths between 50 and 100 km. The outboard tip of the arc, the toe of the accretionary wedge, may or may not override the ridge axis.

The models have one frustrating aspect: the key prediction, the depth at which detachment occurs beneath the arc, is not easily determined by either geophysical remote sensing or the geology of exposed sections. van der Beukel's extension of the models to three dimensions is more immediately relevant. Plate failure and ophiolite preservation occur when the colliding ridge is a small part of a much larger plate driven by subduction of older lithosphere elsewhere. This explains the occurrence of young ophiolites in southern Chile. It remains to be seen whether ridge–trench collisions have a large or small role in preserving ophiolites globally. ☐

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CELL BIOLOGY

Phospholipid transfer market

James E. Rothman

PHOSPHOLIPID-transfer proteins (originally termed 'exchange proteins') have the remarkable capacity to exchange lipid molecules between membranes without altering their own total lipid content. In the two decades or so since they were discovered[1], these enigmatic molecules have posed a puzzle which, with the remarkable report by Bankaitis *et al.* on page 561 of this issue[2], is now partly resolved.

The puzzle is this: why should all eukaryotic cells have in their cytoplasm a battery of different transfer proteins that (at least *in vitro*) are incapable of directing net lipid movement between organelles? It is all the more perplexing because transfer proteins will use virtually any biological membrane or synthetic lipid bilayer as substrate. Could such nonspecific proteins possibly mediate the kinds of vectorial movements of lipids between specific organelles that would be useful in membrane biogenesis and recycling? Apparently, yes, as Bankaitis *et al.* demonstrate. With the surprising identification of one of the phospholipid-transfer proteins of yeast as the product of a secretion gene, *SEC14*, a specific biological function for them has at last been found, most probably the vectorial removal from, or delivery of lipids to, the Golgi apparatus in order to stimulate protein secretion.

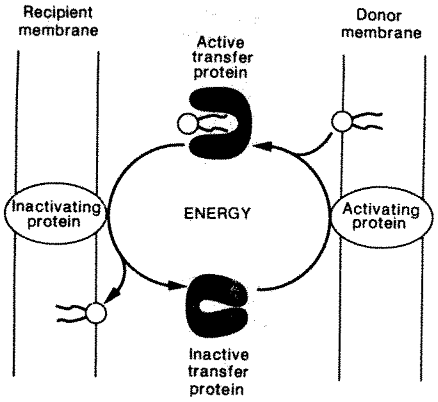
Three kinds of phospholipid-transfer protein, differing in their headgroup specificities, have been purified from mammalian cytosol and their properties investigated[3]. The phosphatidylcholine (PtdCh) transfer protein will catalyse the exchange between bilayers of only PtdCh. The phosphatidylcholine/phosphatidylinositol (PtdCh/PtdIns) protein exchanges both PtdCh and PtdIns. And a nonspecific transfer protein will exchange all species of phospholipids (and also sterols).

All three kinds of transfer protein seem to work by acting as diffusible carriers of one lipid molecule at a time, according to a simple mechanism: when a lipid-laden transfer protein encounters a membrane surface, it discharges the lipid into the outer leaflet of the bilayer, and does not dissociate until its lipid-binding site is once again full. No net transfer of lipid between membranes is possible with this mechanism, and only the leaflets of membranes facing the cytoplasm are affected. Also, no energy is needed for this exchange process. In the case of the PtdCh/PtdIns transfer protein, the unusual dual headgroup specificity for two dissimilar phospholipids results from a single lipid-binding site that may be occupied either by PtdCh or by PtdIns, but not by both at the same time. This enables the protein to achieve a net transfer of PtdCh or PtdIns between membranes without affecting total lipid content when two membranes initially differing in their PtdCh:PtdIns ratio are mixed.

To test whether transfer proteins have any essential function in cell biology, Dowhan and colleagues[4] undertook to purify the PtdIns/PtdCh transfer protein from baker's yeast. They obtained a cytosol-derived protein of relative molecular mass 35,000 which accounted for most or all of the PtdIns and PtdCh transfer activity of crude yeast cytosol. Using probes derived from a partial amino-acid sequence, they obtained a DNA clone of the corresponding structural gene (termed *PIT1*). Disruption of the *PIT1* gene by homologous recombination was lethal, implying that the PtdCh/PtdIns-transfer protein performs an essential task in cell biology. But what could this be?

The answer has come from the confluence of this work with an independent line of investigation by Bankaitis and colleagues[5], who had cloned and sequenced the *SEC14* gene, also from yeast. The *sec14-1*^{ts} mutant is temperature-sensitive[6], and has an unusual phenotype: at the restrictive temperature, secretion from the Golgi to the cell surface ceases, but transport from the endoplasmic reticulum (ER) into the Golgi continues. The secretion block is accompanied by a massive accumulation of Golgi membranes. Normally, the existence of a Golgi in yeast has to be taken almost as an article of faith, the membranes being sparse and elusive. But *sec14-1*^{ts} cells accumulate Golgi membranes as their principal cytoplasmic organelle.

In their report[2], Bankaitis, Dowhan and coworkers note that the published[4] N-terminal protein sequence of the PtdCh/PtdIns-transfer protein is identical to that of protein product of the *SEC14* gene predicted from the published[5] sequence. They go on to show that the *PIT1* clone, in single copy, will restore growth to *sec14* mutant, and that the nucleotide sequences of the SEC14 and PtdCh/PtdIns-transfer protein clones are identical. Consistent with the hypothesis that the lipid transfer activity of the protein SEC14p is the primary defect in the temperature-sensitive *sec14* mutant, the PtdIns transfer activity of crude cytosol prepared from *sec14-1*^{ts} cells was entirely temperature sensitive *in vitro*. As expected, the PtdCh-transfer activity of yeast cytosol was also thermolabile, but interestingly the PtdCh-transfer activity of sec14p is more thermosensitive



Possible energy-driven cycle resulting in net lipid flow from a lipid donor membrane housing an activating protein (enzyme) to a recipient membrane containing an inactivating protein (enzyme). Inactivating and activating proteins are purely hypothetical.

than its PtdIns-transfer activity.

Bankaitis *et al.*[2] propose that the PtdCh/PtdIns-transfer protein is needed either to remove phospholipids from the Golgi, or alternatively to deliver them to the Golgi, in order to maintain the secretory pathway in an active state. Concerning delivery, they suggest that the transfer of PtdIns to the Golgi from its site of synthesis in the ER by the protein (presumably in exchange for Golgi PtdCh) may be needed for Golgi function. But this seems an *ad hoc* explanation, as it is hard to understand why the existing vesicle flow from the ER to the Golgi would not suffice to provide the Golgi with PtdIns. Nor is there any particular reason to envisage a special role for PtdIns in Golgi transport, as distinct from, say, exit from the ER, because in the *sec14* mutant the first not not the second process is disrupted.

Thus, at first glance, a role for the transfer protein in removing phospholipid from the Golgi would seem the more attractive. The Golgi constantly receives a massive input of lipid in the form of transport vesicles derived from the ER membrane during secretion. Wieland *et al.*[7] have estimated that, in animal cells, half of the phospholipid of the ER is transported into the Golgi in these vesicles every 10 minutes; because the total surface area of the ER is 5–10 times that of the Golgi, this means that there must be a highly active mechanism for removing most of the phospholipid from the Golgi every minute or two and returning it back to the ER. Any disruption of this mechanism would lead to a swift increase in the surface area of the Golgi, as is observed in the *sec14* mutant, because of the accumulation of unrecycled phospholipid bilayer. A block in vesicular transport within the Golgi (but not from ER to Golgi) might well be a secondary consequence of this accumulation, as the activities of many membrane-bound enzymes are affected by lipid content and composition, and undoubtedly a number of (as yet unknown) membrane proteins are required to act in concert to achieve vesicular transport.

Wieland *et al.*[7] also note that unidirectional protein transport from the ER to the Golgi using an unselective, bulk-flow mechanism might require that much of the necessary recycling of phospholipids proceeds by a non-vesicular mechanism, and they raise the possibility that phospholipid-transfer proteins may provide this route. The new findings of Bankaitis *et al.*[2] are in striking, if only superficial, agreement with this prediction, which if correct implies that other transfer proteins must carry phospholipids other than PtdCh and PtdIns back to the ER. There would then also have to be some sort of flip–flop mechanism in the Golgi membrane to provide the luminal leaflet with access to the transfer proteins for recycling.

Whatever the role in secretion of phospholipid transfer by the PtdCh/PtdIns-transfer protein proves to be, the results of Bankaitis *et al.*[2] point to a biological specificity for the Golgi that has no basis in the behaviour of the pure transfer protein. This strongly implies that there are other portions of a lipid-transfer machinery that have been missed, and that the diffusible transfer proteins operate together with compartmentalized membrane proteins to provide the directionality and specificity needed for an organized traffic pattern in which there is a net non-vesicular flow of lipids between sites such as the ER and Golgi.

For example (see figure), in the case of the recycling of phospholipid from Golgi to ER, the ER might contain a membrane protein that binds the soluble transfer protein and causes it to discharge its bound lipid, while releasing the transfer protein in a reversibly modified (inactive) form in which its lipid-binding site is empty. When the inactive but still diffusible transfer protein later encounters a specific activating protein in the Golgi membrane, it would be reactivated and then load up with Golgi phospholipid for another cycle. Either the inactivation or the activation step (or both) would require the input of energy, the ultimate purpose being to enable the transfer protein to do the key thing it cannot do by itself: dissociate empty from a membrane. □

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DAEDALUS

The magic bullet

THE great era of comic films established the custard pie as an item of ballistics rather than food. Daedalus is now reviving this charming fantasy. He points out that modern custard, essentially a suspension of corn starch in water, can have strongly non-linear viscosity. It yields to small loads, but can lock almost solid under strong or sudden impact forces. It would therefore make an interesting bullet: capable of delivering a heavy blow, but flowing freely after the impact.

Now ballistic impact has recently been used as a way of getting foreign DNA into cells. Tiny gold or tungsten spheres are coated with the DNA and fired into the target tissue; some of the cells survive the assault and accept the DNA. Daedalus reckons that small droplets of DNA-loaded custard would be even better. Hardened momentarily by the impact, they would penetrate the cell, and then lapse into liquid once more. DNA and nutrient starch would be distributed round the cell without further trauma. Such a relatively gentle injection technique might even be scaled up sufficiently for medical use. Vaccines and other medicaments could be fired through the skin of those patients who seriously don't like needles. Problems of syringe-sterility would vanish too.

The high speed of the pharmaceutical custard droplets would even let them be delivered from some distance away. Daedalus had amusing visions of a whole queue of patients standing with their right sleeves rolled up while the doctor sprayed them with ballistic vaccine like a medical St Valentine's day massacre.

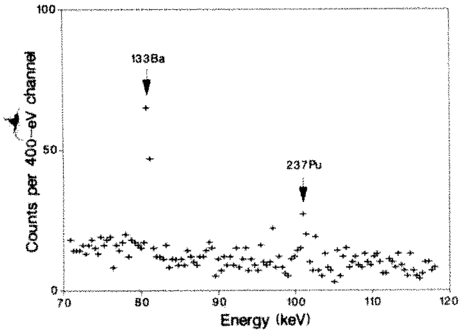
He then recalled how hampered modern policing is by the lack of appropriate weapons. Against rioters, terrorists, hijackers and so on the traditional truncheon is ineffective, while military firearms are too lethal and indiscriminate. But a suitable ballistic custard, loaded with anaesthetic and formulated to be fired from a specially adapted rifle, could solve the whole problem. The aerodynamic forces of flight would probably fragment it into a tight cloud of droplets. On hitting the victim, each droplet would momentarily harden, penetrating his clothing and skin. The resulting minor wound, a mere pin-prick in itself, could deliver a powerful pharmaceutical blow. A liquid missile has such a high density that even a small droplet could carry enough anaesthetic to send a determined aggressor rapidly to sleep.

The custard bullet will be warmly welcomed by police marksmen. At last their lonely burden of responsibility will be lifted. They can hit the innocent with the guilty, knocking out all concerned, then collect up the entire unconscious assembly and leave the apportionment of blame and injury to the courts. David Jones

Leukaemia risk and plutonium

SIR—Müller in Scientific Correspondence[1] suggests that a selective retention of actinides by the male gonads may induce leukaemia in the children of people whose work involves processing these materials. It is commonly assumed for the purposes of radiological protection that most plutonium entering the blood migrates to the liver and skeleton, with only 0.035% being deposited in the testes[2]. This value is based on the observed behaviour of plutonium in various animal species and on concentrations in tissues that were removed at autopsy from three human subjects injected with ^{239}Pu citrate 5–160 days before death from pre-existing disease[3].

The low uptake is further supported by our recent investigations following intravenous injection of ^{237}Pu as citrate in a healthy volunteer aged 62 years. This isotope, decaying by electron capture with a half-life of 45.3 days[4], is a valid metabolic tracer for the long-lived α-emitting isotopes of Pu, despite its much greater specific activity[5]. It was produced by α-irradiation of uranium, which also generated low levels of the α-emitting isotopes[6]. The injection of 1.4 kilobecquerels of ^{237}Pu led to an acceptable estimated effective dose equivalent of 0.3 millisieverts, of which more than 99% arose from the impurities. The main object of our experiment was to study patterns of excretion, clearance from blood, and hepatic and skeletal uptake of Pu isotopes.



Gamma-ray spectrum recorded with a semiconductor detector viewing the testes, showing a well-defined peak from skeletal ^{133}Ba (81 keV) but only a weak response from ^{237}Pu (101 keV).

The assessment of gonadal uptake was conducted inside a heavily shielded room, 28 days after the injection, using a high-purity germanium detector 50 mm in diameter and 20 mm thick, whose proximal planar surface was 5 mm behind the beryllium entrance window. The subject supported himself on hands and knees, with thighs separated sufficiently to allow location of the window within 5 mm of the posterior surfaces of the scrotum. Local close-fitting graded shielding (1 mm cadmium + 2 mm lead) around the curved surfaces of the crystal housing reduced interference from skeletal ^{237}Pu. Part of the γ-ray spectrum recorded during an initial 20-min measurement is shown in the figure, in which most of the response arises from skeletal ^{133}Ba, injected 2½ years previously in a study of normal bone mineral metabolism; deposition of barium in the testes is negligible (1.7×10^{-5} of body content[7]). The ^{133}Ba is responsible for the prominent peak at 81 keV, superimposed on the continuum of scattered radiation from other lines (mainly 356 keV) in its spectrum. The principal photon emissions from ^{237}Pu are the Kα_1 X-rays at 101 keV; there is no well-defined feature at this energy in the figure but the response in the range 100–102 keV exceeds that in the adjacent continuum by 0.92 ± 0.26 (s.d.) counts per min per keV. This elevation cannot be ascribed solely to ^{237}Pu in testes because the response from ^{133}Ba shows that discrimination against skeletal deposits was incomplete.

A second 20-min measurement was therefore made on the same day, with minor postural changes, intended to reduce irradiation of the unshielded window by skeletal activity without impairing detection of ^{237}Pu in the testes. On this occasion, the elevation was only 0.31 ± 0.21 counts per min per keV.

A crude calibration, with estimated accuracy ± 30%, was effected with a mock testicle, conforming roughly to the dimensions given for reference man[7] and containing a solution of ^{237}Pu standardized relative to the injected quantity; on that basis the smaller elevation corresponded to a gonadal deposit of 0.10 ± 0.07%. Clear evidence of ^{133}Ba (81 keV) persisted in the second spectrum, at about 50% of its intensity in the first, and so this estimate must exaggerate the true deposition in our subject. We conclude that there is nothing to invalidate existing assumptions[2] concerning testicular uptake of plutonium.

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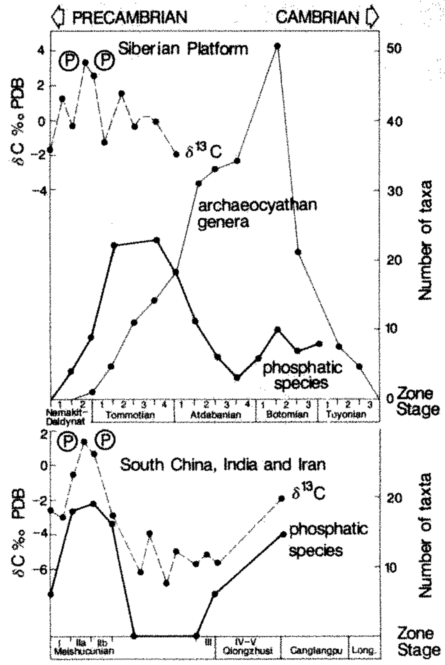
Nutrients in the early Cambrian

SIR—The Precambrian/Cambrian boundary interval is marked by explosive evolution of invertebrate taxa, including the first biomineralized skeletons and by major carbon isotope excursions. A comparison of recent data from each of these lines of evidence in Siberia and south China to Iran (see figure) implies that evolutionary patterns may have been related to shifts in nutrient supply. An early phase of rising productivity and phosphogenesis was accompanied by the initial diversification of phosphatic skeletons. The explosive evolution of oligotrophic sponges with calcitic skeletons, and increasing depths of bioturbation in the sediment, occurred during a later phase of nutrient depletion.

Studies of carbon-isotope stratigraphy across Asia[1] have prompted this question of nutrient supply (especially phosphorus) to the oceans. Changes in δ^{13}C can be related to patterns of primary productivity and nutrient supply in modern lakes and oceans. In the Nemakit–Daldynian stage, these isotopes show an interval of increasingly heavier δ^{13}C (raised productivity), rapidly replaced by lighter isotopes (lowered productivity) close to the base of Tommotian stage in Siberia, and at a similar horizon from south China to Iran. As major phosphogenic events straddle this turning point (P in the figure), huge fluxes in nutrient supply may have occurred. Sedimentological data suggest this high primary productivity took place in shallow carbonate lagoons, perhaps fed with phosphorus by evaporative reflux from the open ocean. Productivity seems to have declined after the burial of phosphorites across Asia and during each of the great transgressions of the early Cambrian. This decline may have been related to reduced circulation within saline, stratified water masses that flooded the shelves.

Recent ecological observations indicate that biological innovations during the Cambrian explosion may have further depleted nutrient supply. For example, greater depth of burrowing, increased grazing of cyanobacterial and algal mats, the production of larger faecal pellets and larger phytoplankton cells all enhance the removal of nutrients and carbon into sediments[2]. Deep bioturbation was not extensive until about Atdabanian times[3].

This model of nutrient glut followed by nutrient depletion is consistent with the contrasting histories of phosphatic skeletons and archaeocyathan sponges through the early Cambrian. The former were phosphophilic, associated with more open-marine, eutrophic conditions. At low latitudes, phosphatic species declined in diversity after the initial phosphogenic stages of the Cambrian explosion[4], while



Contrasting diversity of oligotrophic archaeocyathan genera and 'phosphophilic' phosphatic skeletal species, compared against published carbon isotopes curves from Siberia and south China. P, phosphogenic events.

archaeocyathans diversified explosively[5]. The latter were seldom associated with phosphatic shells or phosphorites, and a preference for oligotrophic conditions may be implied by analogy with recent reefal biotas plus evidence for close associations with cyanobacterial mounds. Symbiotic recycling of phosphorus, nitrogen and organic compounds is inferred.

The peak of archaeocyathan diversity coincided with maximum transgression during the early Cambrian, and declined during a eustatic regression[5]. A return to more phosphogenic conditions and heavier stable isotopes during and after this demise raises important questions about the role of nutrient excess in evolution, like that currently destroying coral reefs.

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Erratum

In the Scientific Correspondence "The law beats Maxwell's demon" by Paul A. Samuelson (*Nature* **347**, 24–25; 6 September 1990) the equation

$$D[\dot{x}, 1/2] = \dot{x} \text{ for } \dot{x} < 0$$

should have read

$$D[\dot{x}, 1/2] = -\dot{x} \text{ for } \dot{x} < 0.$$

□

HIV mutation rate

SIR—The human immunodeficiency virus (HIV) evolves at a rate about a million times as great as that of eukaryotic DNA genomes[1]. This ratio is reflected in the replication accuracies of the corresponding enzymes: the typical error rate of DNA polymerization is somewhere around 10^{-10} and 10^{-9}; the measured average error rate per site for the HIV-1 reverse transcriptase is between 10^{-4} and 10^{-3} (refs 2,3). This implies that the reverse transcriptase makes on average 1–10 errors during the replication of the HIV genome (10^4 bases). This high mutation rate might be an important factor for the virus to escape destruction by the immune system.

An interesting feature of HIV-1 infections is the high genetic variability found in virus populations. Sequential virus isolations from the same infected patients have shown that mutation *in vivo* is rapid: many related but distinguishable mutants evolve during chronic infection[1,4–6]. The selection pressure exerted by the immune response might represent the main force to generate this genetic variability.

The existence of neutralizing antibodies against HIV has been well documented[7,8]. The immunodominant loop (V3 loop), a region of about 30 amino acids within the viral envelope protein gp120, mediates most of the neutralization phenomena in infected humans. This part of the envelope protein seems to mutate rapidly in infected patients. Immunological selection (propagation of new resistant mutants in the presence of specific neutralizing agents) has been observed *in vitro*[9]. The generation of new neutralization resistant mutants might result from a single point mutation in the envelope gene[10]. This production of escape mutants by antigenic drift has also been reported for other lentiviruses.

It seems to be essential for the virus to change its immunodominant epitopes as fast as possible. An obvious question is: what is the optimal mutation rate that maximizes the probability to produce escape mutants?

If the average replication accuracy of the reverse transcriptase is denoted by q, the probability that replication of the whole genome is done without error is given by $Q = q^n$, where n is the total length of the genome. For HIV, $n \sim 10^4$ for one single strand. Let m be the number of sites within the genome such that a mutation in at least one of these sites results in the production of an escape mutant (m might be the length of the immunodominant loop or so, but the particular value for m will not influence our result, as long as m is much smaller than n, which is clear). The probability to obtain an escape mutant (the probability of obtaining a mutant with no errors in $n - m$ sites and at least one error in the m sites) is given by $P = q^{n-m}(1 - q^m)$. This probability has a maximum for $q^* = (1 - m/n)^{1/m}$ which is well approximated by $q^* = 1 - 1/n$. This result is independent of m. Therefore, the optimal mutation rate is given by $e^* = 1 - q^* = 1/n$, which is for HIV around 10^{-4}.

This result could be shifted slightly to higher values if we consider the effect of neutral sites in the genome. Additional mutation in the neutral sites will not change the fitness of an escape mutant. If r is the fraction of neutral sites in the whole genome, then $P = q^{n(1-r)-m}(1 - q^m)$ and $e^* = 1/n(1-r)$. If r lies somewhere between 0 and 0.9, then e^* is found between 10^{-4} and 10^{-3}.

My argument is based on the assumption that the human immune response against HIV favours variation in some parts of the viral genome. If this is true, then there is an optimal mutation rate which maximizes the probability of producing new resistant mutants due to errors in viral replication. This optimal mutation rate is in good agreement with the measured replication accuracy of the HIV-1 reverse transcriptase.

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Radiating bodies

SIR—H. Aspden (*Nature* **347**, 25; 1990) describes a procedure for driving sensible energy from a cooler body to a hotter body, in contravention of thermodynamics. This is done by the use of a mirror to focus radiation from the cooler body A onto the surface of the hotter body B.

This procedure will fail. If the only radiating bodies involved are the bodies A and B, then body B will radiate heat back to body A faster than A radiates to B, so bringing the two bodies to the same temperature, as required by thermodynamics. It would be necessary in practice to enclose both A and B by additional surfaces, but in order that these should make no net contributions to the energy exchanges between A and B, these too would have to be at the same temperature as A and B.

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Trace-element fractionation in plumes and the origin of HIMU mantle beneath the Cameroon line

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The lavas of the Cameroon line display a lead isotope anomaly at the continent/ocean boundary which can be attributed to a fossil mantle plume, the diminishing lateral effects of which can be recognized as far as 400 km to either side. The high $^{206}Pb/^{204}Pb$ ratio—characteristic of the mantle endmember known as 'HIMU'—seems to arise from U/Pb fractionation accompanying melt migration following emplacement of the plume in the upper mantle 125 Myr ago.

MODELS for the genesis of ocean island basalts (OIB) can generally be divided into those invoking direct partial melting of plumes that have risen from below a thermal boundary layer separating the lower mantle from the convecting upper mantle (the latter generally thought to be the source of mid-ocean ridge basalts (MORB))[1-3], and those invoking preferential melting of relatively enriched components which are heterogeneously dispersed through the convecting upper mantle[4-7]. Isotope variations in the mantle are also commonly modelled in terms of different proportions of endmember components. The most clearly defined of these is characterized by very radiogenic Pb and unradiogenic Nd relative to Sr and has been variously referred to as the 'St Helena type' by White[8], the 'LoNd' type by Hart *et al.*[9] and the 'HIMU' type by Zindler and Hart[10]. Magmas of this nature (called HIMU in this paper) have been recognized in St Helena[11], Tubuaii[12-14], Mangaia[12,13], the Cameroon line[15,16], the Cape Verdes[17] and the Oslo Rift[18]. The HIMU component possesses special significance because it also represents the most extreme case of the 'lead paradox'[19] (the lead isotopic compositions of OIB and MORB imply that, relative to bulk Earth, their sources have time-integrated high U/Pb ratios, whereas a mantle source that has yielded melts should be characterized by low U/Pb because U is believed to be more incompatible than Pb). Explanations for the lead paradox generally, or for the HIMU component specifically, have included preferential partitioning of lead into the core for a significant period after accretion[20-22], recycling of ancient oceanic crust[2,8,12,13,17,23], delamination of sub-continental lithosphere[9,24,25], metasomatism by CO_2-rich fluids[13,26], preferential recycling back into the mantle of upper continental crust with the lower continental crust representing a repository of unradiogenic lead[27], and finally the possibility that lead is in fact more incompatible than uranium in mantle melting[28,29].

Studies of the volcanic rocks of the Cameroon line have played a useful part in constraining models for the genesis of OIB because of the unusual independent space-time constraints that can be applied to models of magma genesis[7,15,30,31]. Here we use these space-time constraints to demonstrate that HIMU characteristics were produced relatively recently in an upper-mantle plume as the time-integrated effect of trace-element fractionation between silicate minerals and melt, with U more incompatible than Pb.

The Cameroon line

The Cameroon line is a 1,600-km Y-shaped chain of volcanic centres and plutons extending northeast from the island of Pagalu in the Atlantic Ocean to the continental interior of west Africa (Fig. 1). There are 12 main volcanic centres, the products of which range in age from 30 Myr to the present[7]. In addition, there are 17 pluton complexes in the continental sector, which range in age from 65 to 30 Myr (refs 32, 33). It has been shown by isotope dating that there has been no systematic change in the focus of magmatism during this entire period[7]. Because the African plate has moved with respect to hotspots during the past 65 Myr (refs 34, 35), it is clear that the magmatic activity

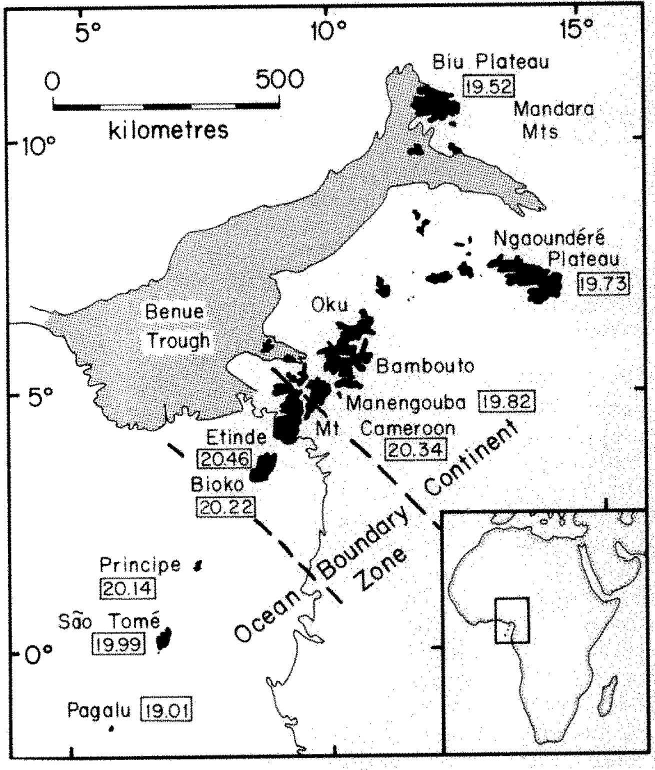

FIG. 1 Map of the area of the Cameroon line and the Gulf of Guinea showing the distribution of the main Cenozoic volcanic centres and the definition of the continent/ocean boundary region. The mean $^{206}Pb/^{204}Pb$ of the < 10 Myr lavas is given for each locality. Data from Table 1 and refs 15 and 16.

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TABLE 1 Isotope and selected chemical data

Volcanic centre	Sample number	Age (Myr)	Lithology	Rb (p.p.m.)	Sr (p.p.m.)	Nd (p.p.m.)	$^{87}Sr/^{86}Sr$	$^{143}Nd/^{144}Nd$	ε_{Nd}	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$
Manengouba	C55	3	Hy-normative basalt	36	636	46	0.70316 (3)	0.512957 (7)	+6.2	19.794	15.609	39.36
Manengouba	C69	1	Trachybasalt	62	788	60	0.70315 (2)	0.512923 (8)	+5.6	19.709	15.613	39.35
Manengouba	C51	1	Alkali basalt	37	757	46	0.70308 (2)	0.512929 (31)	+5.7	19.920	15.611	39.52
Manengouba	C59	1	Alkali basalt	37	945	56	0.70307 (1)	0.512904 (10)	+5.2	20.135	15.665	39.87
Manengouba	C72	1	Alkali basalt	43	918	54	0.70303 (1)	0.512867 (20)	+4.5	20.182	15.694	39.87
Manengouba	C70	1	Basanite	42	733	43	0.70300 (1)	0.512940 (9)	+5.9	19.634	15.625	39.27
Manengouba	C56	1	Hy-normative basalt	16	480	27	0.70299 (1)	0.512959 (10)	+6.3	19.572	15.652	39.30
Manengouba	C68	1	Hy-normative basalt	38	463	38	0.70298 (2)	0.512986 (13)	+6.8	19.795	15.635	39.50
Mt Cameroon	C201	0	Basanite	22	661	42	0.70339 (1)	0.512723 (7)	+1.7	19.983	15.620	39.84
Mt Cameroon	C201D	0	Diopside megacryst	1	74	8	0.70336 (2)	0.512758 (9)	+2.3	20.110	15.640	39.61
Mt Cameroon	C25	0	Basanite	36	989	70	0.70335 (1)	0.512777 (7)	+2.7	20.354	15.658	40.15
Mt Cameroon	C5	0	Tephrite	51	1,202	81	0.70328 (1)	0.512781 (8)	+2.8	20.375	15.655	40.15
Etinde	C24	0	Nephelinite	87	2,401	180	0.70337 (1)	0.512782 (2)	+2.8	20.455	15.662	40.31
Etinde	C150	0	Melanephelinite	89	1,054	85	0.70337 (2)	0.512800 (6)	+3.2	20.289	15.642	40.08
Etinde	C131	0	Hauyne nephelinite	349	2,825	86	0.70334 (1)	0.512809 (7)	+3.3	20.470	15.665	40.28
Etinde	C154	0	Nosean leucite nephelinite	201	6,553	41	0.70333 (1)	0.512835 (7)	+3.8	20.522	15.663	40.34
Etinde	C152	0	Hauyne nephelinite	114	2,395	142	0.70331 (1)	0.512804 (8)	+3.2	20.489	15.666	40.31
Bioko	FP32	0	Tephrite	61	1,199	79	0.70343 (1)	0.512769 (8)	+2.6	20.368	15.667	40.21
Bioko	FP1	0	Alkali basalt	26	726	47	0.70325 (1)	0.512839 (8)	+3.9	20.032	15.652	39.86
Bioko	FP23	0	Alkali basalt	26	685	55	0.70323 (2)	0.512843 (8)	+4.0	20.298	15.691	40.05
Bioko	FP38	0	Alkali basalt	25	647	43	0.70319 (1)	0.512860 (7)	+4.3	20.044	15.646	39.80
Principe	P16	6	Trachyphonolite	314	11	17	0.71092 (2)	0.512815 (9)	+3.5	20.224	15.674	39.80
Principe	P39	6	Phonolite	271	79	17	0.70378 (2)	0.512940 (8)	+5.9	20.259	15.677	39.80
Principe	P40	6	Phonolite	216	240	23	0.70328 (1)	0.512906 (8)	+5.2	20.285	15.673	39.82
Principe	P12	6	Tristanite	180	1.115	36	0.70322 (1)	0.512922 (10)	+5.5	20.226	15.680	39.86
Principe	P13	6	Tristanite	185	1,188	37	0.70301 (2)	0.512865 (20)	+4.4	20.237	15.701	39.93
Principe	P41	6	Trachyphonolite	174	484	27	0.70300 (1)	0.512930 (7)	+5.7	20.190	15.684	39.82
São Tomé	ST54	3	Trachyphonolite	213	468	20	0.70332 (2)	0.512853 (30)	+4.2	20.128	15.674	39.87
São Tomé	ST93	3	Hy-normative basalt	8	1,174	87	0.70320 (1)	0.512889 (10)	+4.9	20.064	15.666	39.79
São Tomé	ST72	3	Basanite	46	1,130	67	0.70318 (1)	0.512919 (6)	+5.5	19.997	15.683	39.72
São Tomé	ST43	1.3	Trachyte	129	463	46	0.70316 (1)	0.512937 (7)	+5.8	20.060	15.658	39.69
São Tomé	ST106	3	Alkali basalt	29	836	51	0.70315 (2)	0.512899 (21)	+5.1	19.944	15.653	39.56
São Tomé	ST100	3	Trachybasalt	139	1,204	39	0.70312 (1)	0.512925 (9)	+5.6	20.014	15.659	39.68
São Tomé	ST73	3	Tephrite	64	1,281	79	0.70307 (1)	0.512953 (7)	+6.1	20.074	15.720	39.80
São Tomé	ST110	3	Phonolitic tephrite	114	1,128	52	0.70299 (1)	0.512980 (10)	+6.7	20.124	15.691	39.84
São Tomé	ST109	3	Basanite	29	824	44	0.70299 (2)	0.512998 (7)	+7.0	19.954	15.676	39.57

Concentrations determined by X-ray fluorescence at University of Edinburgh. Isotope data determined at the Radiogenic Isotope Geochemistry Laboratory (University of Michigan) using techniques outlined elsewhere[55]. Errors refer to least significant digits and are 2σ mean run precisions. All isotopic ratios measured using a VG Sector multicollector mass spectrometer. Nd and Sr isotopic compositions were measured by multi-dynamic peak switching. Pb isotope data determined using static multicollection except C201D, measured with a Daly detector and ion counting. We obtain $^{87}Sr/^{86}Sr$ of 0.710242 (8) (2σ mean, $N=13$) for NBS987, and $^{143}Nd/^{144}Nd$ of 0.511855 (8) ($N=18$) for the La Jolla Nd standard. $^{87}Sr/^{86}Sr$ ratios are normalized to $^{86}Sr/^{88}Sr=0.1194$, and $^{143}Nd/^{144}Nd$ ratios are normalized to $^{146}Nd/^{144}Nd=0.7219$ with power-law fractionation corrections. Pb isotope data are corrected for fractionation and discrimination with a factor of 0.101% per a.m.u., based on measurements of the NBS981 standard. $\varepsilon_{Nd}=\{[(^{143}Nd/^{144}Nd)_{sample}/(^{143}Nd/^{144}Nd)_{bulk\,Earth}]-1\}\times10^4$. Bulk Earth $^{143}Nd/^{144}Nd=0.512638$, $^{147}Sm/^{144}Nd=0.1966$.

cannot be explained by conventional plume models (as have been advanced[36] to explain the Hawaiian hotspot trail, for example). Fitton and co-workers showed[7,30] that the average trace-element compositions of basalts in the Cameroon line were the same in continental and oceanic sectors, and argued that they were therefore derived from sub-lithospheric depths. A recent isotope study revealed, however, that lavas from the continent/ocean boundary region had relatively radiogenic lead, implying a genetic link with the lithosphere[15].

The isotope anomaly

Table 1 shows Nd, Sr and Pb isotope data for young (<10 Myr) samples of basalt, basanite, nephelinite and more evolved rocks from volcanic centres close to the continent/ocean boundary. The three volcanic centres at the continent/ocean boundary (Bioko, Etinde and Mt Cameroon) are distinctive, with radiogenic Pb and unradiogenic Nd for a given Sr isotopic composition relative to the 'mantle array' (Figs 2 and 3). In these respects the data are similar to the compositions observed for Cape Verdes, St Helena, Mangaia and Tubuaii[12-14,17], and therefore of the HIMU mantle type[10]. The anomaly straddles the continent/ocean boundary and is therefore not lithospheric contamination in the normal sense. This is supported by the data for diopside megacryst C201D and its host lava (C201), which have the least radiogenic Pb and Nd of the Mt Cameroon samples (Table 1). Slight contamination by old lithospheric components is a likely cause of such an effect (and for this reason these samples are ignored in further discussion) but clearly could not explain the overall very radiogenic Pb of the continent/ocean boundary lavas. Although the Pb isotope anomaly is most apparent in the three continent/ocean boundary volcanic centres, its effect can be observed in a progressively decreasing manner in the mean Pb isotopic compositions of younger lavas to either side of this zone (Fig. 1). Older Cameroon line lavas at a given locality tend to have less radiogenic lead[15]. Therefore to demonstrate clearly the magnitude of the geographical effect only analyses of young (<10 Myr) lavas are included in Table 1 and Fig. 1.

Plume models

The existence of a lead isotope anomaly in young lavas associated with an older lithospheric boundary underlines the inadequacy of conventional plume models for the Cameroon line (as with many rift-related magmatic provinces[37]) because the geometrical coincidence between a plume from the lower mantle and the continent/ocean boundary, which has moved with plate motions, would have to be accidental. A plausible explanation for the isotope anomaly is that the chemical and isotope characteristics of the magmas are inherited from the melting and/or assimilation of plume-modified mantle that has remained spatially fixed relative to the lithosphere since the formation of the Gulf of Guinea[15]. This mantle would have been a mixture of asthenospheric mantle, excavated older subcontinental lithosphere, and components from a newly rising plume, which had already been instrumental in the formation of the continent/ocean boundary.

At the time of opening of the south Atlantic, the region occupied by the present continent/ocean boundary in the vicin-

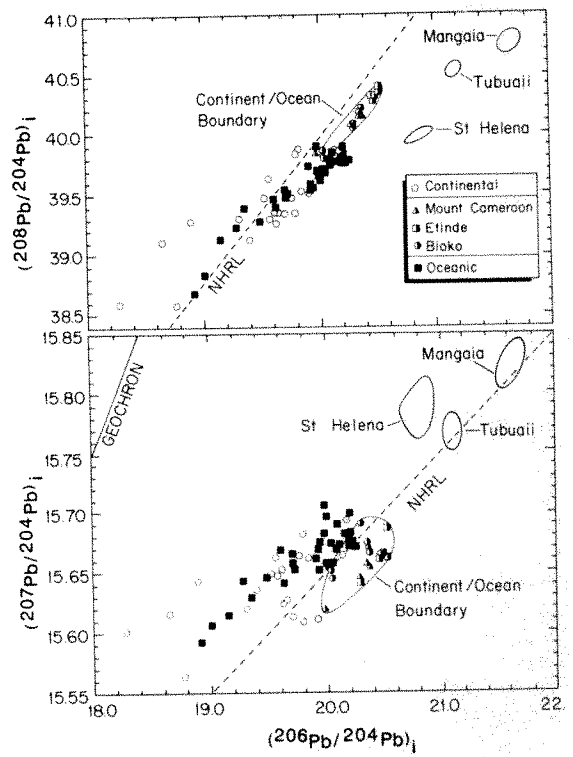

FIG. 2 Plot of initial Pb isotope data for the volcanic rocks of the Cameroon line, in the context of other HIMU (LoNd) types. Data from Table 1, refs 15, 16 and unpublished data for older lavas.

ity of the Gulf of Guinea (Fig. 1) overlay the St Helena hotspot[35]. The idea that the St Helena plume was instrumental in the breakup of Africa, the transfer of HIMU components into the upper mantle and the resultant location of a (diluted) Pb isotope anomaly along the Cameroon line is therefore appealing[15]. The data presented here indicate that such a plume affected the surrounding mantle over a radius of some 400 km. This is similar to the size of plume heads envisaged by White and McKenzie[38].

Schilling and co-workers[39,40] have demonstrated the lateral effects of hotspots on the Mid-Atlantic Ridge and attributed such effects to large-scale lateral flow towards the ridge. In the case of the Cameroon line it is clear that unless the relationship between the hotspots and the continent/ocean boundary is accidental rather than genetic, the general symmetry and apex of the hypothesized plume has been preserved in its original location in the uppermost mantle for 125 Myr. The Cameroon line data cannot be explained by models of redistribution of heterogeneities by large-scale lateral flow in the asthenosphere.

The generation of plume-like anomalies

Despite the apparent elegance of a fossil-plume model and the general similarities between St Helena and Cameroon isotopic compositions, there are problems with this interpretation. The Pb isotope data for the St Helena volcanic rocks are in fact distinct from those of the Cameroon line, and the differences cannot easily be reconciled by hypothesizing different proportions of the same plume component mixed with other Cameroon line compositions. The St Helena volcanic rocks display lower $^{208}Pb/^{204}Pb$ and higher $^{207}Pb/^{204}Pb$ (Fig. 2). The κ_{Pb} values (the time-integrated Th/U ratios deduced from the Pb isotope data[41]) for the continent/ocean boundary samples are consistently 3.9 to 4.0, compared with the values of ~3.7 for St Helena[41]. Furthermore, although showing the same effect of displacement below the 'mantle array', the Nd and Sr isotopic compositions do not lie on a mixing trajectory from the other Cameroon line data towards the field for St Helena (Fig. 3).

A significant feature of the lead isotope data for the continent/ocean boundary samples is that the range in $^{207}Pb/^{204}Pb$ is relatively uniform between different volcanic centres and displays no special distinction from the data for the rest of the Cameroon line; it is only the $^{206}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ ratios that are especially high (Fig. 2). This is illustrated in Fig. 4 where the different centres can be seen to define a series of sub-parallel arrays on a plot of $^{207}Pb/^{204}Pb$ against $^{206}Pb/^{204}Pb$. The slopes of these arrays correspond to apparent ages of roughly 1 Gyr. Such parallel arrays cannot be easily explained as offsets produced by different degrees of mixing between two heterogeneous components with a similar range in $^{207}Pb/^{204}Pb$ (although clearly this could have enhanced the scatter) because each volcanic centre seems to be relatively restricted in its range of $^{206}Pb/^{204}Pb$. Neither can they represent a variety of mixing lines between a radiogenic lead component with high $^{207}Pb/^{204}Pb$ and a range of different components with less radiogenic and variable $^{206}Pb/^{204}Pb$ ratios because the plot of $^{208}Pb/^{204}Pb$ against $^{206}Pb/^{204}Pb$ displays a tight collinear trend for virtually all of the Cameroon line samples (Fig. 2*a*). The offsets between different volcanic centres displayed in Fig. 4 are most simply explained as the result of relatively recent variable enrichment in the U/Pb and Th/Pb ratios. This has the effect of producing correlated offsets in $^{206}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$, with little change in $^{207}Pb/^{204}Pb$, because of the much longer half-lives of ^{238}U and ^{232}Th relative to ^{235}U. Such a process is easily modelled as shown in Fig. 4, assuming that the last major fractionation event took place at 125 Myr when the Gulf of Guinea (hence continent/ocean boundary) formed. The lead isotope data themselves merely indicate young trace-element fractionation; it is the coincidence and inferred genetic link with the continent/ocean boundary that form the basis for proposing the exact timing as 125 Myr. The general magnitudes of $^{238}U/^{204}Pb$ and $^{232}Th/^{238}U$ (approximately equal to Th/U) ratios required to generate the range of modelled lead isotopic compositions are also indicated on Fig. 4. How realistic these values are can be assessed in part by comparisons with measured U/Pb ratios for the lavas themselves. Table 2 provides new isotope-dilution data for U, Pb, Sm and Nd in lavas from the three continent/ocean boundary volcanic centres. The average measured $^{238}U/^{204}Pb$ ratios are generally similar to the modelled values in Fig. 4 and they increase in the order Bioko, Mt Cameroon, Etinde, as predicted by the model.

Implicit in this model is the generally held assumption (recently challenged by Meijer and co-workers[28,29] who have argued the opposite as an explanation for the lead paradox)

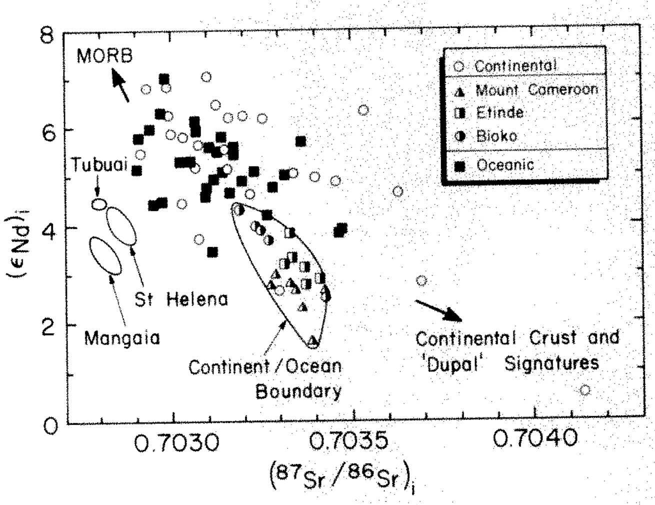

FIG. 3 Plot of initial ε_{Nd} against initial $^{87}Sr/^{86}Sr$ for volcanic rocks of the Cameroon line, in the context of other HIMU (LoNd) types. Data from Table 1, refs 15, 16 and unpublished data for older lavas.

TABLE 2 Sm, Nd, U and Pb concentrations

Locality	Sample number	Sm (p.p.m.)	Nd (p.p.m.)	^{147}Sm/^{144}Nd	U (p.p.m.)	Pb (p.p.m.)	^{238}U/^{204}Pb
Bioko	FP1	9.67	45.6	0.1282	1.07	2.76	25.7
	FP38	8.79	40.5	0.1311	0.775	2.54	20.2
Etinde	C24	25.1	17.6	0.08613	5.68	7.66	49.8
	C150	14.3	89.6	0.09662	2.45	3.84	42.6
Mount Cameroon	C201	7.72	43.0	0.1085	1.25	2.41	34.3
	C25	11.9	68.9	0.1043	2.13	3.75	38.0

Concentrations determined by isotope dilution.

that U is a more incompatible element than Pb in mantle melting. This can be substantiated using the Cameroon line data. Figure 5*a* shows that there is a general positive correlation between lead isotopic composition and measured ^{238}U/^{204}Pb ratio of the more primitive Cameroon line basalts, the slope of which is consistent with a model of young growth of radiogenic lead in the mantle. This relationship suggests that the ^{238}U/^{204}Pb ratio of the lavas is a function of the ratio in the source. Figure 5*b*, *c* shows that the U concentration increases, and the Sm/Nd ratio decreases, with increasing U/Pb ratio, consistent with U being more incompatible than Pb in these magmas. The simplest explanation for a relationship between U/Pb, Nd/Sm and ^{206}Pb/^{204}Pb ratio is that these ratios are correlated in the sources of the magmas. These data, together with those for other basalts, support the view[42] that U is more incompatible than Pb in mantle melting and that the fractionation in U/Pb ratios (as with Sm/Nd ratios) is dominantly controlled by silicate mineral/melt equilibria, rather than by sulphide or metal phases (see ref. 29).

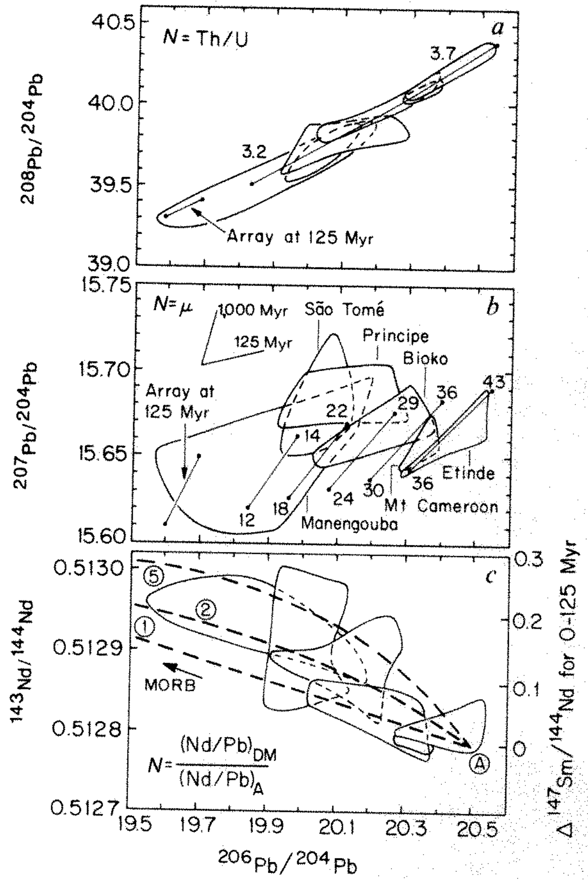

FIG. 4 Pb and Nd isotope data for young (<10 Myr) lavas of the Cameroon line divided according to volcanic centres. The Pb isotope differences between the volcanic centres are explicable if there has been relatively recent fractionation of the U/Pb ratios which will have a greater effect on the ^{206}Pb/^{204}Pb ratio than the ^{207}Pb/^{204}Pb ratio. The lines in *a* and *b* are model Pb isotope arrays produced from an initial linear array at 125 Myr (shown at the left) using the different ^{238}U/^{204}Pb and ^{232}Th/^{238}U values indicated. The model assumes that the ^{238}U/^{204}Pb and ^{232}Th/^{238}U values are proportional to each other and to variations in the ^{238}U/^{204}Pb and ^{232}Th/^{238}U values that produced the original array. *c* shows the relationship between the Pb and Nd isotopic compositions and the considerable ^{147}Sm/^{144}Nd variation necessary to account for the Nd isotope variations purely by radioactive decay over the past 125 Myr. An explanation for the large variation in Nd isotopic composition is that the magmas farther away from the continent/ocean boundary include components from depleted (MORB-like) sources. This is modelled with mixing curves constructed for different values of *N* (circled). Data from Table 1, and refs 15 and 16.

A consequence of the inverse relationship between U/Pb and Sm/Nd fractionation is that increased source U/Pb ratios toward the continent/ocean boundary should be accompanied by decreased Sm/Nd ratios and therefore relatively unradiogenic Nd as shown in Fig. 3. Figure 4 shows the relationship between ^{206}Pb/^{204}Pb ratios and the Nd isotopic compositions of the young volcanic rocks of the Cameroon line. The general trends of the data are consistent with our model, but for the variations in Nd and Sr isotopic composition to be generated entirely by radioactive decay over 125 Myr requires source variations of 0.2 in both ^{147}Sm/^{144}Nd and ^{87}Rb/^{86}Sr. Given the range in U/Pb, Rb/Sr and Sm/Nd measured in the spectrum of OIB and pristine MORB glasses[15,42,43], the modelled source parent/daughter ratios are not impossible, although extreme, particularly for Sm/Nd. The most likely explanation for this is that the lavas from farther away from the continent/ocean boundary have inherited components from more depleted mantle with high Nd/Pb and Sr/Pb ratios such as MORB sources. This explanation is supported by the modelled curvature of the data arrays toward a depleted mantle component with high Nd/Pb (Fig. 4). Such a model is further supported by the relatively unradiogenic lead isotopic compositions of the more distant Cameroon line volcanic centres of Pagalu, Biu and Ngaoundéré[15].

Plume differentiation and interaction

Recent attempts to model the fluid dynamics of plumes and thermals[44-48] have shown that such bodies may rise by a combination of shear flow and entrainment of the surrounding mantle, resulting in incorporation and circulation of components from the ambient mantle in the plume head itself. As a result, the compositional outline of the plume head may develop a 'doughnut shape', with a core of contaminated material. This has the interesting effect that the mantle in the core of the head should not be as enriched as that occupying the region immediately to either side (assuming that the plume is trace-element enriched relative to the ambient mantle)—which should produce a widely spaced pair of anomalies to either side of the plume axis, quite unlike the pattern shown in Fig. 1. We propose instead that the magmas are derived by remelting of a fossil plume, and that the isotope anomaly observed towards the central portions of the Cameroon line, reflects the time-integrated effect of trace-element fractionation during melt migration in the plume head as it cooled after emplacement at 125 Myr. We suggest that the Cameroon line magmas are currently derived from a zone in

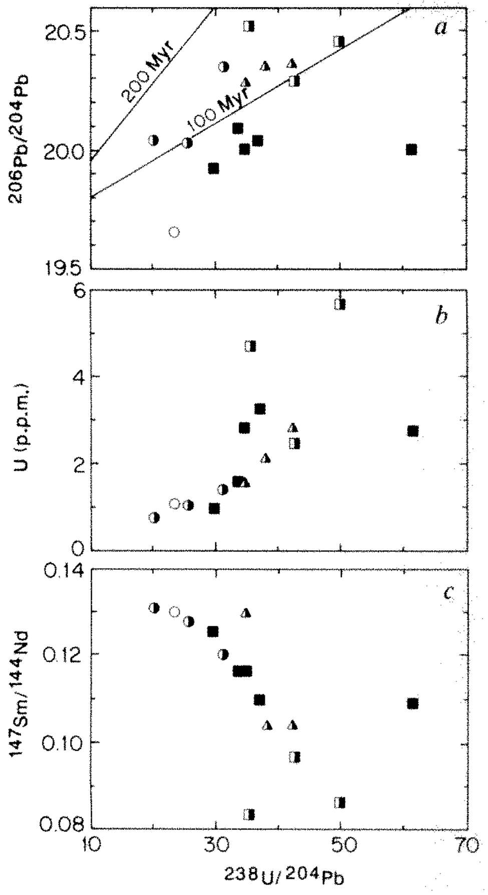

FIG. 5 Relationship betweeen the ^{238}U/^{204}Pb and *a*, ^{206}Pb/^{204}Pb, *b*, U concentration and *c*, ^{147}Sn/^{144}Nd for the young (<10 Myr) Cameroon line lavas with >4 wt% MgO from the volcanic centres in Table 1. These relationships are easily explained if U is more incompatible than Pb and U/Pb (like Sm/Nd) is controlled by silicate solid/liquid partitioning in the magma sources. The one oceanic sample with anomalously high ^{238}U/^{204}Pb is a nephelinite from Principe. Data from Tables 1 and 2, and refs 15, 16.

the upper portions of the fossil plume, enriched by basaltic melts that percolated upward during compaction of the head (Fig. 6). The degree of trace-element enrichment (hence U/Pb ratio) in this zone, would then increase laterally towards the centre, as a function of the vertical thickness of relatively enriched mantle (the flattened plume head) through which the melts migrated during plume compaction. At the margins, where the flattened plume is thinnest, the source regions are dominated by more depleted mantle (Fig. 6), as reflected in the change in Nd, Sr and Pb isotopic compositions towards either end of the Cameroon line, culminating in the highly depleted isotopic compositions observed for centres like Pagalu.

Trace-element fractionation

The Pb isotopic compositions for the three continent/ocean boundary centres imply source U/Pb minima of 0.152 for Etinde and 0.033 for Mt Cameroon, based on the differences with Bioko, assuming closed-system evolution over 125 Myr. The Th/U ratio of the source of these three volcanoes can be estimated at ~3.3 (from the slope of the data in Fig. 2*a*). Although this is probably a slight overestimate[49] it is consistent with the modelling in Fig. 4, and is intermediate between the Cameroon line κ_{Pb} values (3.9–4.0) and the κ_{Th} values of MORB (2.5) (ref. 41). These U/Pb and Th/U estimates, together with the model estimates shown in Fig. 4, provide evidence that the upper-mantle sources are more enriched than MORB sources (in contrast to some models for OIB[7]).

The minimum U/Pb ratio measured for the lavas can be compared with that estimated for the sources based on time-integrated differences, to derive a value for the maximum fractionation in U/Pb ratio (4) during production of the continent/ocean boundary magmas. The U/Pb fractionation required to generate the range in lead isotopic compositions is less than five. U/Pb fractionations in this range can easily be achieved with fractional melting[50,51], and bulk (solid/liquid) distribution coefficients for U and Pb that are within a factor of about six of each other, assuming a constant melt fraction in contact with solid of 0.01, and fractions of melt removed of between 0.01 and 0.10. The U/Pb fractionations hypothesized for both 125 Myr and during the melting that produced the young Cameroon line magmas, are therefore reasonable given the range of experimental silicate mineral/melt U and Pb partition coefficients[52,53], and do not require special CO_2 metasomatism or phases other than silicates.

The reasonably common occurrence of basalts with HIMU isotope characteristics is evidence that the fractionation processes that took place during melting in the mantle under the Cameroon line may not be unusual. Obviously such fractionations do not have to be recent as in the case of the Cameroon line (the St Helena ^{207}Pb/^{204}Pb ratios cannot be explained by the model of recent fractionation presented here). Nevertheless an important implication of the large range in parent/daughter ratios measured and modelled here is that they will generate mantle that is extremely heterogeneous isotopically if allowed to evolve over long periods of time (gigayears). Although such an effect is well illustrated by storage in the sub-continental lithosphere[54], the relative homogeneity of MORB and to a lesser extent OIB compositions[43] implies that heterogeneities produced from small amounts of partial melting are destroyed or greatly diluted in the long-term cycling of the convecting mantle. In these respects our data are consistent with the evidence for short residence times for lead in the upper mantle presented by Galer and O'Nions[41].

Although the Cameroon line plume was apparently already relatively enriched when it was introduced into the upper mantle at 125 Myr, the high κ_{Pb} values for the Cameroon line are difficult to reconcile with models that explain this enrichment in terms of recycling of uranium-enriched (low Th/U) altered oceanic crust into the deep mantle (such as is more readily applied to the data for St Helena, for example). If trace-element fractionation by melting in the mantle produces Nd–Sr isotope data that are below the mantle array and can be taken to the

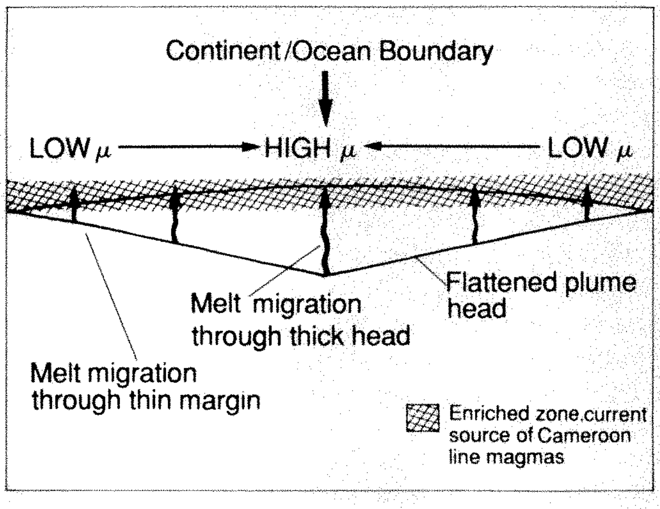
Continent/Ocean Boundary
LOW μ
HIGH μ
LOW μ
Flattened plume head
Melt migration through thick head
Melt migration through thin margin
▨ Enriched zone,current source of Cameroon line magmas

FIG. 6 Cartoon illustrating model for source enrichment following plume emplacement at 125 Myr.

extreme of explaining all HIMU components, the case for a distinctive isotope signature for ancient recycled oceanic crust may need re-evaluating. Conversely the best explanation for the mantle array itself may be recycling, rather than melt fractionation[10,11]. Regardless of these possibilities, this study shows that HIMU mantle can simply represent the time-integrated consequence of storage of small-degree partial melts in the upper mantle and, contrary to recent suggestions, the lead paradox is not resolvable simply in terms of U and Pb partitioning during melting. □

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Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene *pit-1*

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Mutations at the mouse dwarf locus (*dw*) interrupt the normal development of the anterior pituitary gland, resulting in the loss of expression of growth hormone, prolactin and thyroid-stimulating hormone, and hypoplasia of their respective cell types. Disruptions in the gene encoding the POU-domain transcription factor, Pit-1, occur in both characterized alleles of the dwarf locus. The data indicate that Pit-1 is necessary for the specification of the phenotype of three cell types in the anterior pituitary, and directly link a transcription factor to commitment and progression events in mammalian organogenesis.

ANALYSES of developmental *Drosophila* and *Caenorhabditis elegans* mutants[1–4] suggest models in which sequential activation of a hierarchy of regulatory genes dictates the temporal and spatial patterns of the expression of gene products that define cell phenotypes. Similarly, organogenesis in mammals results from the ordered expression of cell phenotypes and, presumably, is also determined by the sequential activation of regulatory genes. The development of the anterior pituitary gland provides an excellent model system in which to study cell-specific gene activation and the processes by which distinct cell types develop within an organ. The anterior pituitary arises from a placode in Rathke's pouch, derived from the somatic ectoderm[5]. The five phenotypically distinct cell types appear in a stereotypical order with the growth hormone-producing somatotrophs and the prolactin-producing lactotrophs being the last two cell types to appear[1–7].

The cell-specific expression of the rat prolactin and growth hormone genes depends on synergistic interactions between *cis*-active elements[8–16]. A pituitary-specific transcription factor, Pit-1, binds to these elements in both genes and can transactivate prolactin and growth hormone gene promoters both *in vitro* and in cell transfection experiments (refs 17–20, R. Maurer & D. Sharp personal communication), although one group[21] found that Pit-1 binds only to the growth hormone gene promoter. The expression of *pit-1* is initially detected on embryonic days 15 and 16 (e15–16) in the rat, followed by the expression of growth hormone and prolactin on embryonic day 17 (ref. 7). Because *pit-1* expression precedes the expression of prolactin and growth hormone genes, the ontogeny of expression of the gene encoding Pit-1 is consistent with a critical role for this transcription factor in the expression of prolactin and growth hormone genes. In the mature pituitary, Pit-1 protein is detected in somatotrophs, lactotrophs and thyrotrophs[7]. Pit-1 is a member of a large gene family of transcription factors, referred to as POU-domain proteins, characterized by a 60-amino-acid region similar to the classical homeodomain and a 76-amino acid region, referred to as the POU-specific domain (for review see refs 22–24). In *C. elegans*, the POU-domain gene *unc-86* regulates developmental events[25]. These data implicate the POU-domain transcription factors in the regulation of cell-specific developmental events in mammals.

Because a mutation in the *pit-1* gene would be predicted to abolish the expression of growth hormone, this mutation would most probably cause a dwarf phenotype. The Snell dwarf mouse (*dw*) was the first genetically transmitted dwarfism observed in mice, and was characterized as a single autosomal recessive mutation[26,27]. The primary defect in *dw* was first suggested to be intrinsic to the anterior pituitary gland because of the hypoplastic nature of the organ[28,29] and the results of transplantation experiments[30]. Antisera against growth hormone, prolactin and thyroid-stimulating hormone (TSH) failed to detect synthesis of these hormones in the anterior pituitary, and the mature thyrotroph, lactotroph and somatotroph cell types were not observed in Snell dwarfs by electron microscopy[31–34]. The mutation causing the dwarf Jackson (dw^J) mouse was discovered on a C3H/HeJ inbred background, and is allelic to the Snell dwarf mutation[35]. A nonallelic but phenotypically similar dwarf mouse mutation called Ames dwarf (*df*) defines a second dwarf locus[36,37]. The phenotype of both dwarf loci includes the depletion of somatotroph, lactotroph and thyrotroph cell types in homozygotes[31–34]. Several observations suggest the possibility that defects in the *pit-1* gene might result in a dwarf phenotype, including the ontogeny of *pit-1* expression, its expression in the three pituitary cell types that are affected in dwarf mutants, and the capacity of Pit-1 to activate the growth hormone and prolactin gene promoters.

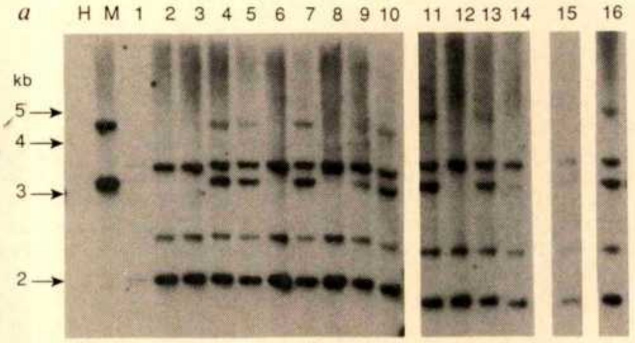
Here we demonstrate a gross structural alteration of the *pit-1* gene in the dwarf Jackson mouse, and a point mutation of an invariant residue in the POU-homeodomain 'recognition helix' that generates a nonfunctional Pit-1 protein in the Snell dwarf. Pit-1 transcripts or protein were not detectable by *in situ* hybridization or immunohistochemistry for mutants with either allele of the *dw* locus.

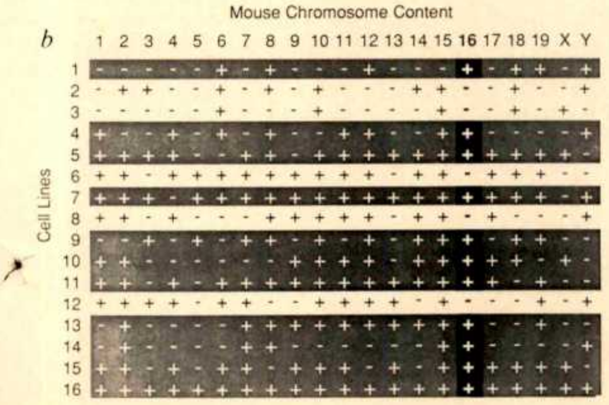
Location of *pit-1* and the *dw* locus

The *pit-1* gene could be the candidate for either *dw* locus on chromosome 16 (ref. 35) or the *df* locus on chromosome 11 (ref. 38). To establish the relationship between these dwarf mice and *pit-1*, the chromosome location of the *pit-1* gene was mapped by somatic cell genetics. The *pit-1* complementary DNA probe hybridized with two *Eco*RI-digested mouse DNA fragments (4.6 kilobases (kb) and 3.2 kb) and three *Eco*RI-digested hamster DNA fragments (3.6 kb, 2.4 kb and 2 kb) (Fig. 1*a*). Among the sixteen hybrid cell lines, all showed characteristic hamster hybridization, and a subset of these cell lines showed mouse-specific hybridization. Figure 1*b* compares the mouse chromosome content of these hybrid cell lines and their hybridization with the *pit-1* probe. Mouse chromosome 16 was present in all the cell lines that showed mouse-specific hybridization, and was absent in those that did not. None of the other mouse chromosomes met this criterion, permitting assignment of the *pit-1* gene to mouse chromosome 16. Because the *dw* mutation was previously mapped to mouse chromosome 16 (ref. 35), this colocalization suggested that the *pit-1* gene was a candidate for the *dw* locus.

A *pit-1* gene RFLP is present in dw^J

To determine whether the *pit-1* gene has undergone a gross structural change, we analysed restriction fragment length polymorphisms (RFLP) of the *pit-1* gene in both alleles of the *dw*




b
Mouse Chromosome Content
Cell Lines

Cell Lines	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	**16**	17	18	19	X	Y
1	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	**+**	-	+	+	-	+
2	-	+	+	-	-	+	-	+	-	+	-	-	-	+	+	-	-	+	-	-	+
3	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	+	-	+	-
4	+	-	-	+	-	+	-	+	-	-	+	+	-	-	+	**+**	-	-	-	-	+
5	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	**+**	+	+	+	+	-
6	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-	-
7	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	**+**	+	+	+	-	+
8	+	+	-	+	-	-	-	+	+	+	+	+	-	+	+	-	+	-	-	-	+
9	-	-	+	-	+	-	+	+	-	+	-	+	-	+	+	**+**	-	+	+	-	-
10	+	+	-	-	-	-	-	-	+	+	+	+	-	+	+	**+**	+	+	-	+	-
11	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	**+**	+	-	+	-	-
12	+	+	+	+	-	+	+	-	-	+	+	+	+	-	+	-	-	-	+	-	+
13	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+	**+**	-	-	+	-	-
14	-	+	-	-	-	-	+	+	-	-	-	-	-	-	+	**+**	-	-	-	-	+
15	+	+	-	+	-	+	+	-	+	+	+	-	+	-	+	**+**	+	+	+	+	-
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**+**	+	+	+	+	+

FIG. 1 Chromosome localization of *pit-1* gene. *a*, DNA blot analysis[45] of the mouse *pit-1* gene in mouse–hamster hybrid cell lines. H. hamster; M. mouse; each number represents a mouse–hamster hybrid cell line. All lines had a hamster restriction pattern (3.4 kb, 2.4 kb and 2 kb) and only some of the lines had mouse-specific hybridization (4.6 kb and 3.2 kb). *b*, Correlation of the mouse-specific hybridization and the mouse chromosome content. '+' indicates that a specific mouse chromosome is contained in that cell line; '–' indicates the absence of the particular mouse chromosome. The grey rows correspond to the cell lines showing mouse-specific hybridization on DNA blot analysis. Mouse chromosome 16 (bold) was present in all the cell lines showing a mouse-specific *pit-1* restriction pattern and was absent from all the cell lines that did not show mouse-specific hybridization.

METHODS. Approximately 10 μg DNA prepared from each of the sixteen different mouse/hamster hybrid cell lines was digested with *Eco*RI, electrophoresed in a 0.8% agarose gel, and transferred to nitrocellulose membranes. An *Eco*RI fragment of rat *pit-1* cDNA that contained most of the N-terminal portion of *pit-1* plus half of the sequence encoding the POU-specific domain[17] was labelled by random priming to a specific activity $>10^9$ c.p.m. μg^{-1}. The filters were hybridized to this probe overnight at 42 °C in 50% formamide/10% dextran sulphate/6 × SSC, and then washed at 60 °C in 0.1 × SSC. The weak hybridization signal in some of the lanes was due to low quantities of DNA. The variation in the relative intensities of mouse versus hamster hybridization among cell lines is due to different percentages of cells within the cell line that contain the mouse chromosome (data not shown).

locus. The *pit-1* gene of Snell dwarf (*dw*) and dwarf Jackson (dw^J) was compared with that of corresponding wild-type strains. In Snell dwarf genomic DNA, no variations in restriction patterns of the *pit-1* gene between *dw* and wild-type strain were observed with any of the five restriction enzymes shown in Fig. 2*a* or three other restriction enzymes (*KpnI*, *SstI* and *XhoI*; data not shown). RFLPs were observed for dwarf Jackson in several restriction enzyme digestions, with patterns differing between dw^J and its wild-type strain (Fig. 2*b*). Because the dw^J allele arose on an inbred genetic background (C3H/HeJ), it was unlikely that the RFLPs result from variations among individual mice within the background strain. The variability of *pit-1* genomic structure among several inbred mouse strains (A/J, AKR/J, C57BL/6J, Balb/cJ, CBA/J, DBA/2J, C3H/HeJ and SJL/J) was also examined by *Eco*RI digestion; all of them had the same restriction patterns (data not shown). The different restriction patterns displayed by dw^J and its wild-type strain indicate that the structure of the *pit-1* gene itself, or a closely linked gene, is altered in dw^J.

The *pit-1* gene is disrupted in dw^J

To characterize further the mutation in dwarf Jackson, the mouse *pit-1* gene was isolated from a genomic library. Restriction mapping of eight positive clones indicated that the complete *pit-1* coding region was represented in two overlapping clones 61 and 32 (Fig. 3*a*). The mapped *Eco*RI sites and the genomic structure are presented in Fig. 3*a*. The six exons, labelled I–VI, encoded amino acids 1–47, 48–72, 73–147, 148–201, 195–221 and 222–291 of mouse Pit-1, respectively (Fig. 4). To map the dwarf Jackson mutation further, *Eco*RI-digested mutant and wild-type genomic DNA were hybridized with the five adjacent *pit-1* genomic *Eco*RI fragments depicted in Fig. 3*a* (probe a contained only half of an *Eco*RI fragment ending at the 5′ end of clone 32). Four of the five probes gave the expected restriction pattern with both normal and dwarf genomic DNA, whereas probe c hybridized to a single 3.2-kb fragment in normal DNA and two fragments (4.6 and 2.4 kb) in dwarf Jackson DNA. These data are consistent with a mutational event that resulted in either an inversion or an insertion of a DNA segment of >4 kb in the *pit-1* gene. On the basis of polymerase chain reaction (PCR) analysis, most of exon III is intact, but the exact break point has not been established. Regardless of which type of rearrangement event has occurred, the results clearly demonstrate that there is an disturbance in the *pit-1* gene in dwarf Jackson, suggesting that mutation of the *pit-1* gene accounts for the dwarf phenotype.

Point mutation in *pit-1* homeobox of *dw* mice.

Because the Snell dwarf did not reveal any altered RFLP bands (Fig. 2), we reasoned that the Snell dwarf mutation was likely to result from a point mutation in the gene. To test this hypothesis, *pit-1* cDNAs were cloned by PCR from normal mouse and Snell dwarf pituitary glands. To clone the normal mouse cDNA, two oligonucleotides corresponding to rat *pit-1* cDNA sequences were used; the first oligonucleotide corresponded to 5′ sense strand noncoding sequences, with the 3′ boundary of the oligonucleotide at the initiator methionine codon ATG; the second oligonucleotide corresponded to 3′ antisense noncoding sequences with its 3′ end encompassing the stop codon TAA. The high degree of similarity between rat and mouse sequences

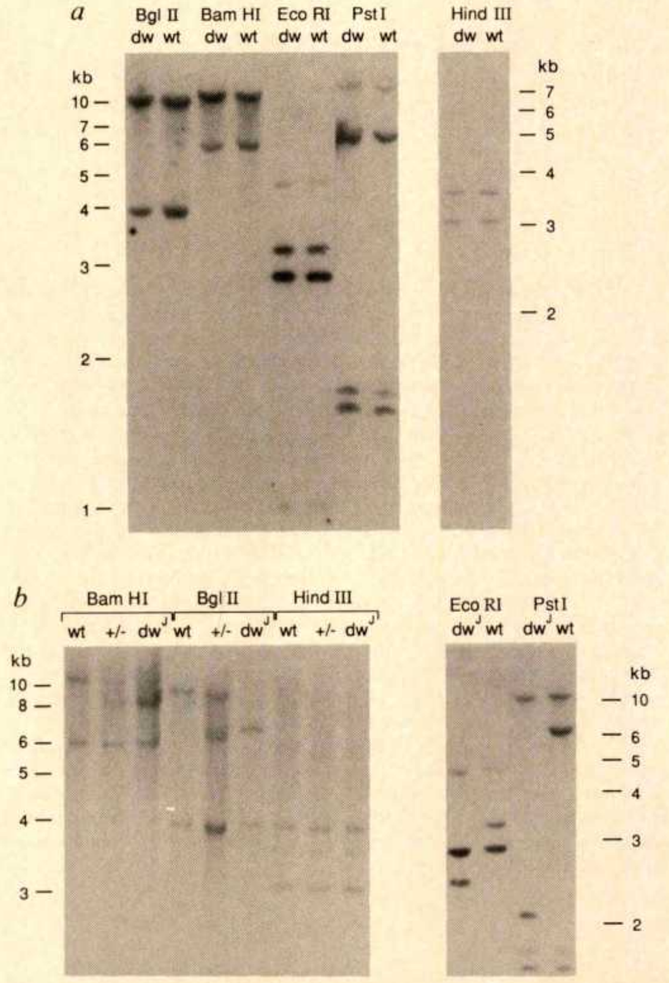


FIG. 2 Restriction analysis of Snell and Jackson *pit-1* genes. *a*, DNA blot analysis of Snell dwarf and wild-type DNA. Abbreviations: *dw*, homozygou Snell dwarf; wt, wild-type strain, Restriction patterns observed in *dw* and its wild-type siblings were similar with all five restriction enzymes. *b*, DNA blot analysis of dwarf Jackson and wild-type DNA. Abbreviations: dw^J, homozygous dwarf Jackson; wt, the wild-type strain (C3H/HeJ); +/− heterozygotes. *Bam*HI, *Bgl*II, *Eco*RI and *Pst* I digestion demonstrated structural changes associated with *pit-1* gene in dwarf Jackson mouse.

METHODS. Genomic DNA was prepared from the dwarf animals and their wild-type strains obtained from Jackson Laboratories (Bar Harbor). The dwarf Jackson mutation arose and is maintained on an inbred background (C3H/HeJ); the Snell dwarf mutation arose in strain of black-silver mice and was maintained on a background strain designated DW/J (distinct from the mutant dw^J)[26,35]. DNA blots were prepared as described in Fig. 1, and probed with a mouse *pit-1* cDNA fragment encompassing the coding region (Fig. 4, nucleotides 60–846), with the exception of those blots containing DNA digested with *Bam*HI, *Bgl*II, and *Hin*dIII in *b*, which were probed with a 1.6-kb rat cDNA fragment[17].

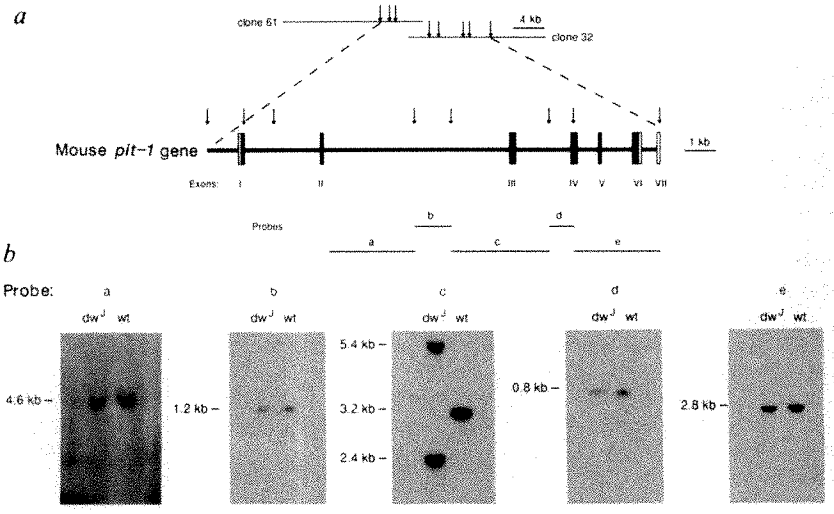
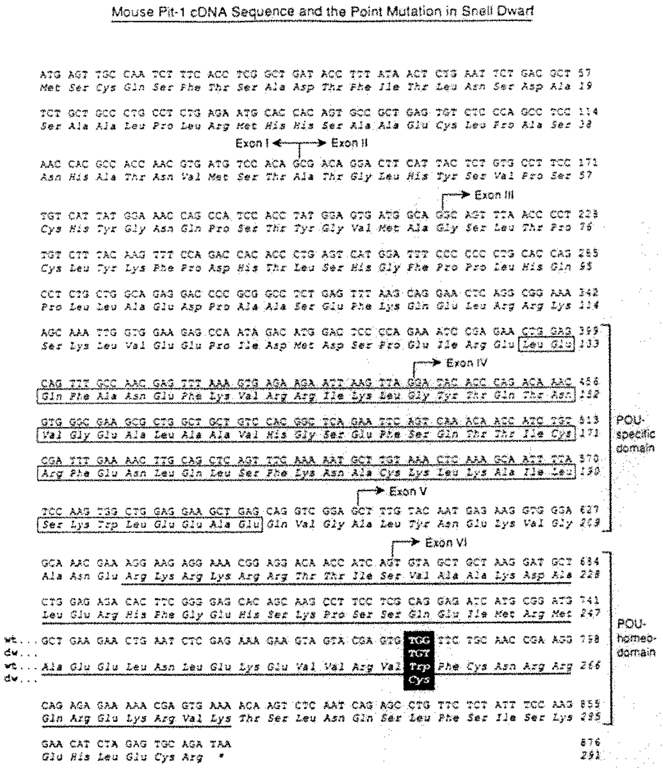


FIG. 3 Mouse *pit-1* genomic structure and the disruption in dwarf Jackson mice. *a*, Schematic representation of mouse *pit-1* gene. Two overlapping mouse λFIXII genomic clones (clone 61 and clone 32) are represented by two straight lines with arrows marking the mapped *Eco*RI restriction sites. All exon-intron boundaries were defined by genomic DNA sequencing[48]. The *pit-1* coding portion between the two dashed lines is enlarged to illustrate the exon-intron structure (see Fig. 4 for detail). The coding and noncoding exons are illustrated by the filled and open boxes, respectively. The *Eco*RI fragments (*a*–*e*) that were used to examine *pit-1* gene disruption in dwarf Jackson mutants are depicted underneath the genomic structure in the corresponding positions. *b*, DNA blot analysis of *pit-1* gene of dw^J mutants. The DNA blots are arranged from left to right corresponding to the probes from 5′ to 3′. Letters *a*–*e* correspond to the letters in *a*; dw^J, homozygote dwarf Jackson; wt, wild-type strain. Only probe c revealed a structural difference in which the 3.2-kb *Eco*RI fragment in the wild type is disrupted in dw^J, resulting in two different fragments, 5.4 kb and 2.4 kb. The estimated sizes of the fragments are placed next to the marks that indicate their positions.

METHODS. Duplicate filter lifts of λFIXII mouse (B6/CBA) genomic library (Stratagene) were hybridized with two sets of oligonucleotides, each containing an oligonucleotide corresponding to N-terminal and C-terminal sequences. Set 1: (sense, nucleotides 58–83) TCTGC-TGCCC-TGCCT-CTGAG-AATGCA; (antisense, nucleotides 824–847) TAGAG-AACAG-GCTCT-GATTG-AGAC. Set 2: (antisense, nucleotides 118–141) TGTGG-ACATC-ACGTT-GGTGG-CGTG; (sense, nucleotides 711–750) TCGCA-GGAGA-TCATG-CGGAT-GGCTG-AAGAA. From 10^6 clones screened, eight primary plaques were positive for both sets of oligonucleotides. A mouse cDNA fragment (nucleotides 27–876), encompassing all four oligonucleotides, was used for secondary screening. On tertiary screening, one plaque hybridized to the N-terminal oligonucleotide probe, and the other seven hybridized only to a C-terminal oligonucleotide probe. Phage DNAs were initially analysed by partial and complete restriction digestions, combined with DNA blot analysis[47] probed by either *pit-1* cDNA fragments or phage T3 and T7 primers that corresponded to sequences in the phage vector λFIXII (data not shown). The overlap between the N-terminal clone and the C-terminal clones was first demonstrated using the end-labelled probe (ref. 49; data not shown). The portion of genomic fragment encompassing the entire *pit-1* coding region was subcloned into plasmid pBKS II(-) (Stratagene). The exon-intron boundaries were determined by sequencing and the distances between exons were estimated by restriction analysis and confirmed by PCR. The Southern blots were carried out as described in Fig. 1, and the genomic DNA was digested using *Eco*RI.


Mouse Pit-1 cDNA Sequence and the Point Mutation in Snell Dwarf

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ATG AGT TGC CAA TCT TTC ACC TCG GCT GAT ACC TTT ATA ACT CTG AAT TCT GAC GCT 57
Met Ser Cys Gln Ser Phe Thr Ser Ala Asp Thr Phe Ile Thr Leu Asn Ser Asp Ala 19

TCT GCT GCC CTG CCT CTG AGA ATG CAC CAC AGT GCC GCT GAG TGT CTC CCA GCC TCC 114
Ser Ala Ala Leu Pro Leu Arg Met His His Ser Ala Ala Glu Cys Leu Pro Ala Ser 38
                                  Exon I <——> Exon II
AAC CAC GCC ACC AAC GTG ATG TCC ACA GCG ACA GGA CTT CAT TAC TCT GTG CCT TCC 171
Asn His Ala Thr Asn Val Met Ser Thr Ala Thr Gly Leu His Tyr Ser Val Pro Ser 57
                                                             ——> Exon III
TGT CAT TAT GGA AAC CAG CCA TCC ACC TAT GGA GTG ATG GCA GGC AGT TTA ACC CCT 228
Cys His Tyr Gly Asn Gln Pro Ser Thr Tyr Gly Val Met Ala Gly Ser Leu Thr Pro 76

TGT CTT TAC AAG TTT CCA GAC CAC ACC CTG AGT CAT GGA TTT CCC CCC CTG CAC CAG 285
Cys Leu Tyr Lys Phe Pro Asp His Thr Leu Ser His Gly Phe Pro Pro Leu His Gln 95

CCT CTG CTG GCA GAG GAC CCC GCG GCC TCT GAG TTT AAG CAG GAA CTC AGG CGG AAA 342
Pro Leu Leu Ala Glu Asp Pro Ala Ala Ser Glu Phe Lys Gln Glu Leu Arg Arg Lys 114

AGC AAA TTG GTG GAA GAG CCA ATA GAC ATG GAC TCC CCA GAA ATC CGA GAA CTG GAG 399
Ser Lys Leu Val Glu Glu Pro Ile Asp Met Asp Ser Pro Glu Ile Arg Glu Leu Glu 133
                                                   ——> Exon IV
CAG TTT GCC AAC GAG TTT AAA GTG AGA AGA ATT AAG TTA GGA TAC ACC CAG ACA AAC 456
Gln Phe Ala Asn Glu Phe Lys Val Arg Arg Ile Lys Leu Gly Tyr Thr Gln Thr Asn 152

GTG GGC GAA GCG CTG GCT GCT GTC CAC GGC TCA GAA TTC AGT CAA ACA ACC ATC TGT 513  POU-
Val Gly Glu Ala Leu Ala Ala Val His Gly Ser Glu Phe Ser Gln Thr Thr Ile Cys 171  specific
                                                                                 domain
CGA TTT GAA AAC TTG CAG CTC AGT TTC AAA AAT GCT TGT AAA CTC AAA GCA ATT TTA 570
Arg Phe Glu Asn Leu Gln Leu Ser Phe Lys Asn Ala Cys Lys Leu Lys Ala Ile Leu 190
                                           ——> Exon V
TCC AAG TGG CTG GAG GAA GCT GAG CAG GTC GGA GCT TTG TAC AAT GAG AAG GTG GGA 627
Ser Lys Trp Leu Glu Glu Ala Glu Gln Val Gly Ala Leu Tyr Asn Glu Lys Val Gly 209
                                              ——> Exon VI
GCA AAC GAA AGG AAG AGG AAA CGG AGG ACA ACC ATC AGT GTA GCT GCT AAG GAT GCT 684
Ala Asn Glu Arg Lys Arg Lys Arg Arg Thr Thr Ile Ser Val Ala Ala Lys Asp Ala 228

CTG GAG AGA CAC TTC GGG GAG CAC AGC AAG CCT TCC TCG CAG GAG ATC ATG CGG ATG 741
Leu Glu Arg His Phe Gly Glu His Ser Lys Pro Ser Ser Gln Glu Ile Met Arg Met 247
                                                                                 POU-
wt... GCT GAA GAA CTG AAT CTC GAG AAA GAA GTA GTA CGA GTG TGG TTC TGC AAC CGA AGG 798  homeo-
dw...                                                     TGT                        domain
wt... Ala Glu Glu Leu Asn Leu Glu Lys Glu Val Val Arg Val Trp Phe Cys Asn Arg Arg 266
dw...                                                     Cys

CAG AGA GAA AAA CGA GTG AAA ACA AGT CTC AAT CAG AGC CTG TTC TCT ATT TCC AAG 855
Gln Arg Glu Lys Arg Val Lys Thr Ser Leu Asn Gln Ser Leu Phe Ser Ile Ser Lys 285

GAA CAT CTA GAG TGC AGA TAA                                                 876
Glu His Leu Glu Cys Arg  *                                                  291
```

FIG. 4 Mouse *pit-1* cDNA sequence; exon-intron boundaries, and the point mutation in the Snell dwarf. Mouse *pit-1* cDNA sequence from ATG to TAA are shown, together with the protein sequence. The cDNA sequence was obtained from mouse pituitary mRNA by PCR, except for the sequence between nucleotides 1–58 and 847–876 that come from the mouse genomic sequence. The codon containing the point mutation found in Snell dwarf cDNA (G-to-T change) is marked by a black box as are the corresponding changes in amino-acid sequence from tryptophan to cysteine. The POU-specific domain is boxed and the POU homeodomain is underlined. The exact exon boundaries are marked by arrows and the numbering of the exons is the same as in Fig. 3.

METHODS. For the isolation of the initial normal mouse *pit-1* cDNA, total RNA, prepared from mouse pituitary by an acid guanidinium-phenol-chloroform method was reverse-transcribed into cDNA (5 μg RNA template; oligo(dT) as a primer), and was then amplified by PCR[49] with degenerate oligonucleotides. Two rounds of amplification were necessary for isolation of enough *pit-1* fragment to clone; both rounds used the same 3′ antisense oligonucleotide that encoded all possible codon combinations for the rat amino acids that correspond to residues 283 to the stop codon. For the first round of amplification, the 5′ sense oligonucleotide encoded all possible codon combinations for the rat Pit-1 amino acids that correspond to residues 1–8; the second PCR included an oligonucleotide corresponding to residues 10–20. PCR-30 cycles (round 1) or 35 cycles (round 2) at 94 °C for 1 min, 50 °C (round 1) or 45 °C (round 2) for 2 min, and 72 °C for 3 min. Complementary DNA was generated as above from the total pituitary RNA of eight homozygote Snell dwarf mice (pituitary glands were a gift of Dr W. Beamer, Jackson Laboratory). The first round of PCR used two oligonucleotides terminating at the ATG start codon (sense, GTTCTCTACTCTCTTGTGGGAATG) or the TAA stop codon (antisense, GTACCGATGGTGTCCGTTCAGAAT). One round of PCR amplification was sufficient to isolate enough fragment to clone from a small segment (<20%) of a normal mouse pituitary, but another round of PCR was necessary to isolate sufficient fragment from eight Snell dwarf pituitaries. The second round of PCR used oligonucleotides that lie within and slightly overlap those used in the first round of amplification (sense, GAATGAGTTGCCAACCTTTCACCT; antisense, AGAATAGACGTGAGT-TCTACGAGG). PCR = 35 cycles (round 1) or 20 cycles (round 2) at 94 °C for 1 min, 50 °C for 2 min, and 72 °C for 3 min. The reaction mixtures were separated on an agarose gel and the DNA fragment of expected size was subcloned and sequenced by the dideoxy chain termination method[48]. The characterization of exon boundaries has been described in Fig. 3.

easily permitted the isolation of *pit-1* from normal mouse pituitaries (Fig. 4). But the oligonucleotides did not amplify a discernable band from Snell dwarf pituitary cDNA. A second round of amplification, using two more internal oligonucleotides, was necessary to isolate Snell dwarf *pit-1* cDNA. Two independent subclones of the PCR product were sequenced, and comparison of the mutant and wild-type sequences revealed a G-to-T alteration in the Snell dwarf *pit-1* cDNA that would convert the tryptophan residue in the POU-homeodomain (Trp 261) to a cysteine residue (Fig. 4). To exclude the possibility of PCR artefacts, oligonucleotides encompassing the mutation were designed on the basis of genomic sequences in exon VI, and one of these oligonucleotides corresponded to sequences that fell outside the cDNA clone; this strategy excluded the possibility of reamplifying any previously isolated cDNA products, thereby avoiding any artefacts due to contamination. PCR products from each of the normal genomic DNA gave the wild-type sequence, but each of the four Snell dwarf PCR preparations showed the G→T mutation of the cDNA clones (Fig. 5*a*, *b*). The mutated tryptophan residue is conserved in organisms from mammals to yeast, and exists in all classes of the POU- and homeodomain proteins (reviewed in refs 2, 39, 40). The importance of this tryptophan residue in the function of Pit-1 is illustrated by the fact that this mutation abolishes binding to a high affinity *pit-1* site (Prl-1P; data not shown), and mutations of this region in Oct-1 (tryptophan, phenylalamine, cysteine residues) also abolish DNA binding[41].

Expression of *pit-1* in *dw*, *dw^J* and Ames dwarfs

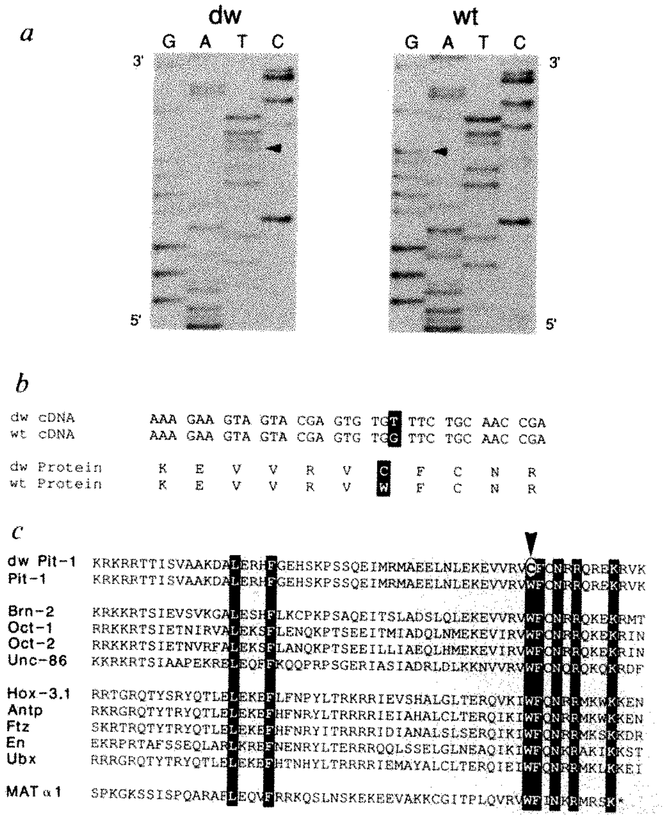
Having characterized mutations of the *pit-1* gene in *dw^J* and Snell dwarfs, we determined using immunohistochemistry and hybridization histochemistry whether the expression of *pit-1* messenger RNA and protein is normal in these mutants. In contrast to the wild-type controls, neither mRNA nor protein were detected in either of the dwarf mice (Fig. 6). The failure to detect *pit-1* mRNA or protein in the dwarf Jackson pituitary indicates that *pit-1* expression was impaired or abolished as a consequence of the gross structural alterations in the gene. The finding that *pit-1* transcripts in Snell dwarf mouse could be amplified only by the PCR, together with the results of the *in situ* hybridization and immunohistochemical studies, suggests that there is a low level of *pit-1* gene expression in the Snell dwarf.

Because an understanding of the effect of the Ames dwa mutation on the expression of *pit-1* would help to elucidate the mechanisms that lead to similar phenotypes for these two dwarf loci, similar experiments were conducted on the Ames dwarf pituitary with virtually identical results (Fig. 6). The lack of detectable *pit-1* gene expression in the phenotypically similar Ames dwarf indicated that the Ames mutation is epistatic to the expression of *pit-1*. Thus, the Ames dwarf locus might be involved in the regulation of *pit-1*, or perhaps, in conjunction with *pit-1*, for the specification and/or maintenance of the three specific pituitary cell types affected by the mutation.

Discussion

The characterization of tissue-specific gene activation in mammalian organ systems has provided a wealth of information about the phenotypes of mature cells, but clear insight into the role of these factors during developments has been hampered by the lack of a genetic animal model system. Here we have shown that both alleles of the *dw* locus result from mutations in the gene encoding the pituitary-specific transcription factor, Pit-1. In Snell dwarfs, a point mutation in the *pit-1* gene alters the functionally critical tryptophan residue that is invariant throughout eukaryotic evolution in the POU- and homeodomain transcription factors. These dwarf genetic models therefore provide the initial genetic evidence for the role of a well characterized transcription factor during mammalian organogenesis.

Because mutations in the *pit-1* gene result in the loss of three pituitary cell types in the *dw* mutants, *pit-1* must activate a part of the pituitary developmental program. The absence of the



```
dw cDNA      AAA GAA GTA GTA CGA GTG TGT TTC TGC AAC CGA
wt cDNA      AAA GAA GTA GTA CGA GTG TGG TTC TGC AAC CGA

dw Protein    K   E   V   V   R   V   C   F   C   N   R
wt Protein    K   E   V   V   R   V   W   F   C   N   R
```

```
dw Pit-1  KRKRRTTISVAAKDALERHFGEHSKPSSQEIMRMAEELNLEKEVVRVCFQNRRQREKRVK
Pit-1     KRKRRTTISVAAKDALERHFGEHSKPSSQEIMRMAEELNLEKEVVRVWFQNRRQREKRVK

Brn-2     KRKKRTSIEVSVKGALESHFLKCPKPSAQEITSLADSLQLEKEVVRVWFQNRRQKEKRMT
Oct-1     RRKKRTSIETNIRVALEKSFLENQKPTSEEITMIADQLNMEKEVIRVWFQNRRQKEKRIN
Oct-2     RRKKRTSIETNVRFALEKSFLANQKPTSEEILLIAEQLHMEKEVIRVWFQNRRQKEKRIN
Unc-86    KKRKRTSIAAPEKRELEQFFKQQPRPSGERIASIADRLDLKKNVVRVWFQNQRQKQKRDF

Hox-3.1   RRTGRQTYSRYQTLELEKEFLFNPYLTRKRRIEVSHALGLTERQVKIWFQNRRMKWKKEN
Antp      RKRGRQTYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKEN
Ftz       SKRTRQTYTRYQTLELEKEFHFNRYITRRRRIDIANALSLSERQIKIWFQNRRMKSKKDR
En        EKRPRTAFSSEQLARLKREFNENRYLTERRRQQLSSELGLNEAQIKIWFQNKRAKIKKST
Ubx       RRRGRQTYTRYQTLELEKEFHTNHYLTRRRRIEMAYALCLTERQIEIWFQNRRMKLKKEI

MATα1     SPKGKSSISPQARAFLEQVFRRKQSLNSKEKEEVAKKCGITPLQVRVWFINKRMRSK*
```

FIG. 5 Confirmation of the point mutation of the highly conserved Trp 261. *a*, Sequence gel of Snell dwarf genomic fragment across the point mutation. The arrows mark the nucleotide change revealed on the sequence gel. Th orientation of the sequence gel is indicated, as well as the nucleotides in each lane. Abbreviations: *dw*, homozygote Snell dwarf; wt, wild-type strain. *b*, Sequence represented in the gel of *a* and the corresponding protein sequence. The mutation, which causes a G-to-T change is highlighted by bold white letters in the black box, as is the resulting change in the amino acid Trp to Cys. *c*, Evolutionary conservation of Trp 261. The amino-acid sequences of different classes of POU homeodomains and classical homeodomains were aligned with the mutated Pit-1 POU homeodomain. The Trp 261 which is mutated to Cys (bold in the circle under the arrow) in Snell (*dw* Pit-1) is one of the seven (white bold letters in the black boxes) amino acids that are invariantly conserved in all the POU-domain and homeodomain proteins. Abbreviations: dw Pit-1, Snell dwarf mouse Pit-1; Pit-1, mouse Pit-1; Brn-2 (ref. 39); Oct-1; Oct-2; Unc-86 (reviewed in ref. 22); Hox 3.1, mouse homeobox gene *Hox* 3.1 product, Antp, *Antennapedia* gene product; Ftz, *fushi tarazu* gene product; En, *engrailed* gene product; Ubx, *Ultrabithorax* gene product (see ref. 2); MATα1, yeast mating type regulatory gene product[40].

METHODS. Two oligonucleotides corresponding to sequences ~150 base pairs, apart, sense oligonucleotide (nucleotides 711–750) and the antisense oligonucleotide CACACATGGCTATCACACGCCAGC, designed on the basis of mouse genomic sequence outside the TAA at which the mouse and rat sequences diverge were used in a PCR of the genomic DNA from four Snell dwarf mutants and two wild-type animals. The amplified fragments were subcloned and sequenced as shown.

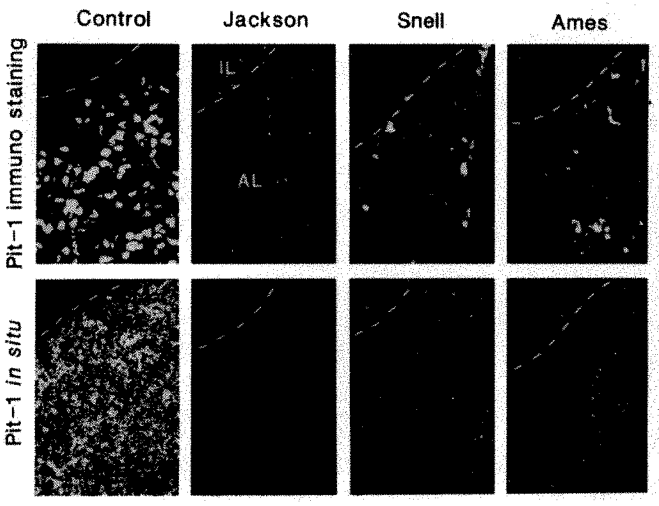


FIG. 6 Pit-1 immunostaining (top) and *in situ* hybridization (bottom) of tissue sections from 3–4 week old control and dwarf mouse pituitaries. In control mice, detectable immunostaining for Pit-1 protein was confined to cell nuclei in the anterior lobe (AL), but was absent in sections from Jackson, Snell and Ames dwarf mice. Small bright structures in the sections from mutant mice were erythrocytes, which autofluoresced orange, and were thus easy to distinguish from the specific Pit-1 immunofluorescence (green due to the fluorescein-conjugated secondary antiserum) when viewed under epifluorescent illumination. *In situ* hybridization specific for *pit-1* mRNA (silver grains appear white in these darkfield photomicrographs) were confined to the AL control mice. In sections from mutant mice. silver grain counts show that background labelling was uniformly distributed over the AL and intermediate lobe (IL); boundaries between IL and AL are indicated by dashed white lines and were determined by viewing a fluorescent counterstain (bisbenzimide, not visible with the illumination used for photography.)

METHODS. The immunohistochemical and hybridization histochemical methods and reagents were as those described in detail elsewhere[10]. Fresh pituitaries were dissected out and fixed immediately in 4% paraformaldehyde in 0.1 M borate buffer (pH 9.5), plus 10% sucrose for 12 h. Then, 5 μm-thick, frozen sections were cut on a cryostat. A 450-nucleotide [^{35}S]-complementary RNA probe directed towards the 5′ end of the *Pit-1* mRNA[10] was used for *in situ* hybridization at a concentration of 5×10^6 c.p.m. ml^{-1}.

three cell types corresponds to the cells in which *pit-1* expression is observed in the normal pituitary gland[7]. The analyses of *dw* mutants prove that *pit-1* is required for the specification of somatotroph and lactotroph phenotypes, which includes the activation of growth hormone and prolactin gene expression. These data argue against the suggestion that a subset of lactotrophs are derived by way of a Pit-1 independent pathway[42], and that the role of *pit-1* is restricted to the specification of somatotroph phenotype[14,21]. The evidence provided by the genetic dwarf locus clearly demonstrates a broader role of *pit-1* in pituitary organogenesis.

Although the specific function of *pit-1* in thyrotrophs is not so far clear, the disappearance of TSH cells in *pit-1*-defective dwarf animals implies an important role for *pit-1* in the specification and/or maintenance of thyrotroph phenotype. The appearance of thyrotrophs (βTSH) before the detectable expression of the *pit-1* gene may indicate that *pit-1* is required for cell survival and proliferation of TSH-producing cells, possibly by the regulation of cell machinery that responds to trophic factors. But it is possible that *pit-1* expression occurs at levels below the limits of detection before βTSH expression, and is required for the specification of the thyrotroph cell phenotype.

Homeodomain proteins in *Drosophila*, *C. elegans* and mouse have important developmental roles[1-3,43]. Mutations of the POU-domain protein encoded by *unc-86* in *C. elegans* affect several cell lineages throughout the body, or block the progression from immature to mature cell phenotypes[4,25]. In the pituitary, the progression from immature cells to mature somatotrophs and lactotrophs could involve a pool of presomatotroph stem cells[44-46]. The observation of putative immature cells with histological characteristics of both somatotrophs and lactotrophs in Snell mice[33,34] suggests a role for *pit-1* in the developmental progression of stem cells to a mature pituitary cell type.

The hypoplastic nature of the dwarf pituitary indicates that cell proliferation and/or survival are also a component of the programme specified by *pit-1*. These data are consistent with a role of *pit-1* in the regulation of other genes—such as those encoding growth factor receptors or receptors for hypothalamic trophic factors—that are required in the proliferation and developmental progression of stem cells to a mature pituitary cell type. By permitting the characterization of gene products absent in hypoplastic dwarf pituitaries, the genetic dwarf model system will allow the full characterization of the developmental programme specified by *pit-1* during pituitary organogenesis. □

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Discovery of an X-ray burst from X2127+11 in the globular cluster M15

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THE nature and evolution of binary X-ray sources in globular clusters are poorly understood. Among the ~10 bright X-ray sources in globular clusters, X2127+11 in M15 is very important because it is the only one for which the optical counterpart, AC211, and orbital period, 8.5 h, are both known[1]. We have detected a type I X-ray burst from X2127+11, which was the only bright X-ray source in a globular cluster from which no burst had been detected. The burst was of long duration ($\gtrsim$150 s) and had a precursor separated from the main peak by ~6 s. This means that the burst was very energetic and that a large photospheric expansion occurred. The observed burst peak luminosity, well above 10^{38} erg s^{-1}, is hard to reconcile with the standard idea that X2127+11 is screened from direct view by an accretion disk. This latter view arises from consideration of the low ratio of X-ray to optical luminosity, which suggests that only a small fraction of X-rays is visible through scattering by an accretion-disk corona around the source[2-4].

We observed X2127+11 in the globular cluster M15 with the large-area proportional counters[5] (LAC) on the Ginga satellite[6]. The observations were carried out on 20–25 October 1988, and the net observation time (excluding the Earth occultation of the source and high-background portions such as south Atlantic anomaly passages) was ~1.0×10^5 s. During the observations the average X-ray flux of X2127+11 was 5×10^{-10} erg cm^{-2} s^{-1} (1–28 keV), and we observed erratic intensity variations of a factor of about three in addition to a clear 8.5-h orbital modulation[7]. We detected an energetic X-ray burst from X2127+11 on 20 October 17:34:50 UT (onset time). We could not observe the end of the burst because the Earth occulted X2127+11.

Although the LAC has a relatively wide field of view (1°×2° full width at half maximum), it is clear that the burst came from X2127+11 because no other known X-ray source was in the field of view and because there was a significant decrease in the persistent X-ray flux between the precursor and the main peak of the burst (Fig. 2). The burst must therefore have come from the source of the persistent emission, X2127+11.

There are two kinds of X-ray bursts[8,9]. Type I bursts are observed from all X-ray burst sources, whereas type II bursts have so far only been observed in the Rapid Burster. Type I bursts are characterized by a temperature decrease and an emission region of approximately constant size during the burst decay. Type II bursts, on the other hand, are characterized by an approximately constant temperature and a decrease in the size of the emission region during the burst decay. It is believed that type I bursts result from the runaway of thermonuclear burning on the surface of a neutron star, whereas type II bursts result from instability of the mass accretion onto the neutron star. In Fig. 1, we show the count rate plotted against time for the burst from X2127+11 in four energy bands. This burst is extremely energetic and the large photospheric expansion distinguishes it from the usual type I bursts. But there was a temperature decrease in the decay of the burst (after 17:36:30) indicated by the difference in the decay timescale between the higher and the lower energy bands, and we therefore conclude that the burst was a type I burst.

The burst had a precursor that preceded the main peak by ~6 s. Such bursts with precursors have been observed from several sources[10,11]. They are extremely energetic, and the precursor has been explained by photospheric expansion as a

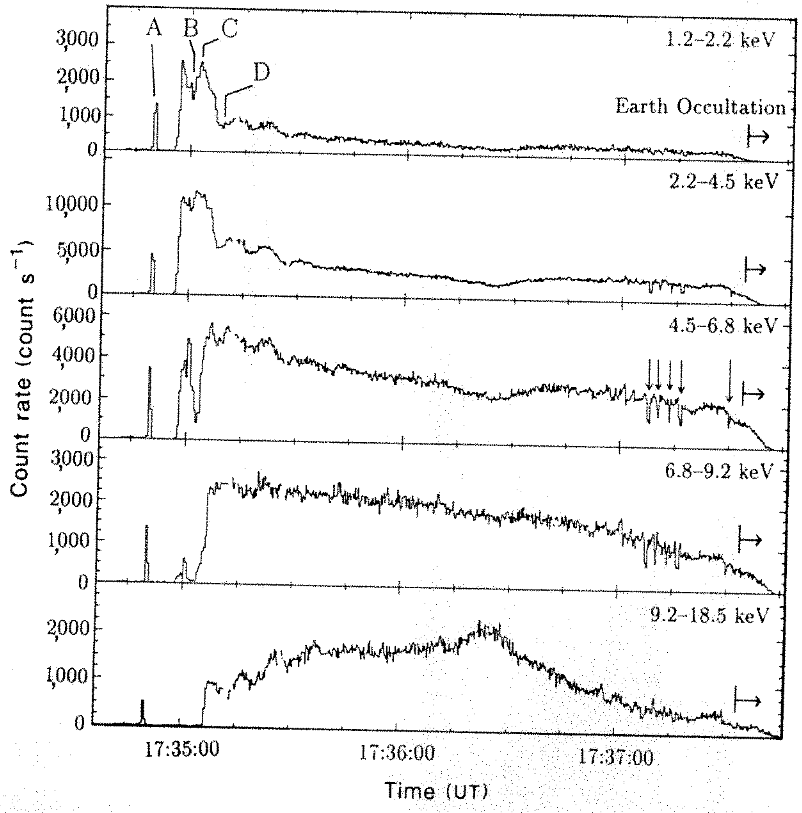
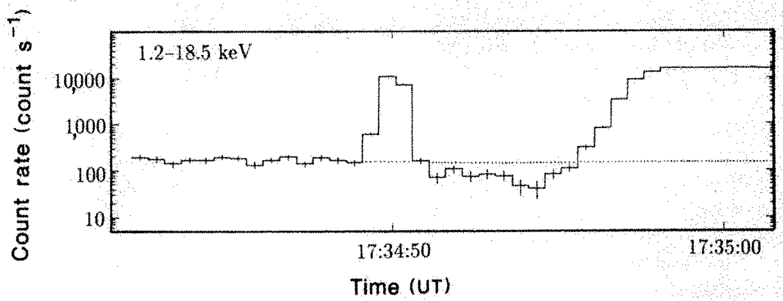


FIG. 1 Plot of the count rate against time of the type I X-ray burst from X2127+11. The observation was made in 48 energy channels (0.5-s time resolution) until just after the peak of the burst and then switched to 12-energy-channel mode (7.8-ms time resolution); the latter data are re-binned to 0.25-s time bin in the figure. Non-X-ray and diffuse-X-ray background have been subtracted, and the data corrected for aspect. The rapid decrease of the count rate after 17:37:35 is an artefact owing to Earth occultation of the source. Five dips are indicated by arrows near the end of the burst. Marks A–D indicate times of the energy spectra shown in Fig. 3*a–d*, respectively.

FIG. 2 Count rate at the onset of the burst. Data are plotted on a logarithmic scale to show the change in the persistent emission between the precursor and the main part of the burst. The persistent emission level before the burst occurrence is indicated by the dotted line. Data have been corrected as in Fig. 1.



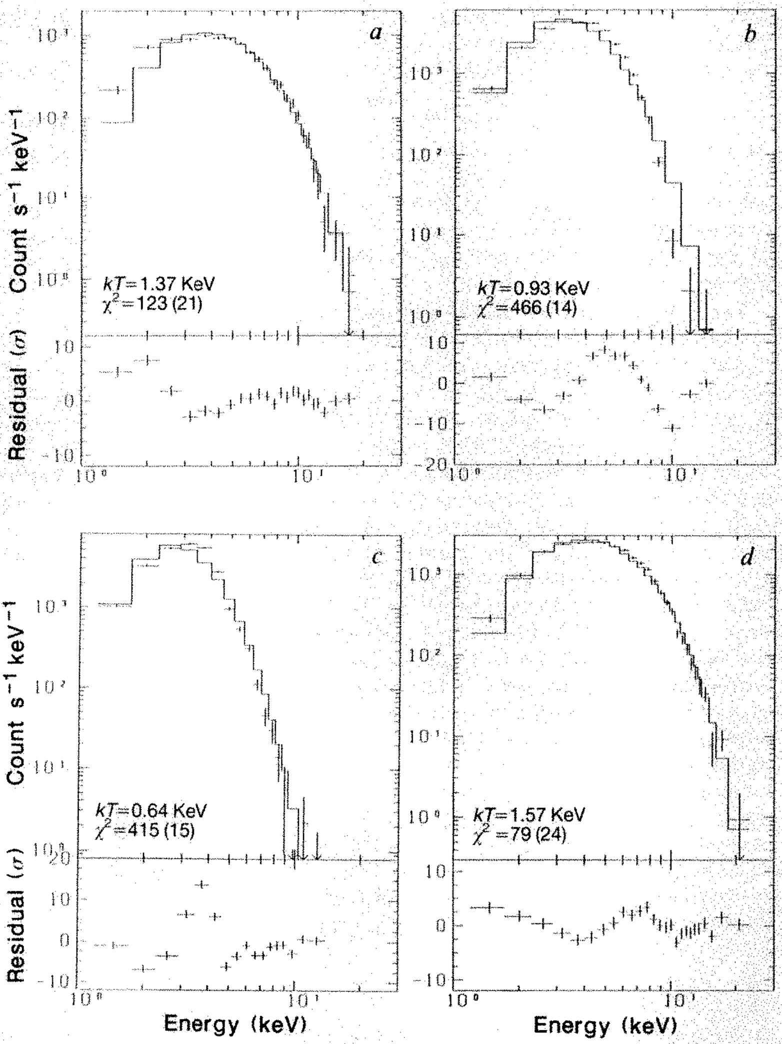
consequence of excessive radiation pressure[12,13]. The precursor corresponds to the burst onset. The luminosity quickly reaches the Eddington limit, and the photosphere of the neutron star expands very rapidly. This reduces its temperature and the bulk of the emission is shifted to lower photon energies outside the X-ray band, forming a precursor. After a few seconds, the excess radiation pressure diminishes and the photosphere begins to contract. As a result, the temperature rises and X-ray emission resumes from the low-energy end.

The burst profile has several notable features: (1) significant X-ray flux between the precursor and the main part of the burst; (2) oscillations of the X-ray flux as the photosphere contracts; (3) five dips towards the end of the burst. The X-ray flux between the precursor and the main part of the burst is shown in Fig. 2. This X-ray flux was about half of the persistent emission before the burst occurred. Although the count statistics during this period are poor, no qualitative change in the spectrum is evident. During this period, the cooled photosphere of the neutron star must have been large enough (more than several hundreds kilometres) to cover the inner part of the accretion disk. As the photosphere is too cool to emit X-rays and the X-ray emission from the inner part of the accretion disk is screened by the photosphere, the origin of the remaining X-rays is unclear.

The oscillations of the X-ray flux have a period of $\sim$10 s and can be seen for several cycles in the course of contraction of the photosphere (Fig. 1). There is a phase reversal between the soft- and the hard-energy bands. Similar oscillations showing an energy-dependent phase reversal have been observed during a big burst from X1608 − 52 (ref. 14). Those oscillations were interpreted as the radial oscillations of an envelope supported by radiation pressure near the Eddington limit[15]. Although the period of oscillation of X2127 + 11 ($\sim$10 s) is much longer than that of X1608 − 52 (0.65 s), there may be a common mechanism.

The five dips (marked by arrows in Fig. 1) near the end of

FIG. 3 Four representative energy spectra of the burst during the photospheric expansion (*a*–*c*) and after contraction of the photosphere (*d*) (times indicated by A–D in Fig. 1). Non-X-ray and diffuse-X-ray background have been subtracted (see text). The best-fit blackbody is shown by histograms. The colour temperature of the blackbody and χ^2 of the fit (degree of freedom in parentheses) are indicated. The energy spectra deviate from the blackbody spectrum during the photospheric expansion (*a*–*c*), but the energy spectrum in *d* is relatively well approximated by a blackbody.



the burst have a duration of about one second and are separated by about three seconds, except for the fifth one. Because the depth of the dips is almost independent of the X-ray energy, the dips, if caused by photoelectric absorption, must be due to clouds that are opaque to X-rays at least up to 10 keV. Furthermore, the size of the cloud is such that it occults only about half of the emission region. Such dips of short duration are unusual, and seem to be different from the transient dips observed from partial eclipsing binaries[16].

The energy spectrum of type I bursts is generally well expressed by a blackbody spectrum with a colour temperature decreasing as the burst decays[9]. Our spectra exhibit significant deviations from blackbody spectra, however, particularly when the best-fit colour temperature is below 1.5 keV. Figure 3 shows four examples of observed spectra (at the times indicated in Fig. 1) together with best-fit blackbody spectra. We subtracted non-X-ray and diffuse-X-ray background from the data because the persistent emission level may have changed during the burst, but the burst was so energetic that the choice of the background had little effect on the spectra obtained. More than a simple hard-X-ray tail is required to explain the deviation of the spectra from a blackbody. By contrast, when the colour temperature is high ($kT > 1.5$ keV), the spectra are relatively well approximated by a blackbody. Such a deviation of the burst spectra from that of a blackbody has not been noted before and its origin is unknown.

The bolometric correction for the determination of the peak flux of the burst is somewhat uncertain because of this deviation from a blackbody spectrum. We therefore integrated the observed flux above 2 keV and corrected only for the energy-dependent detection efficiency, which gives >90% of the total flux when $kT > 1.5$ keV. Thus the luminosity just after the contraction of the photosphere is estimated to be $\sim 5 \times 10^{38}$ erg s^{-1} for the well estimated distance to M15 of 10 kpc (ref. 17). This value is much larger than the Eddington limit for a neutron star of $M = 1.4 M_{\odot}$ and $R = 10$ km. The calculation of the radius of the photosphere and the effective temperature is not straightforward because of the deviation from a blackbody spectrum, general relativistic effects, the difference between effective temperature and colour temperature, and the possible anisotropy of the emission. A detailed discussion can be found in van Paradijs *et al.*[18].

The type I burst from X2127+11 confirms that the compact object of this binary source is a neutron star. The X2127+11/AC211 system has relatively small ratio of the X-ray to optical luminosities, $L_X/L_{\rm opt}$, compared to usual values for low-mass X-ray binaries[2,3,19,20]. A plausible interpretation of the low $L_X/L_{\rm opt}$ value is that the central X-ray source is screened from direct view by the accretion disk (an inclination angle near 90°) and that only a small fraction of the X-rays is observable after scattering by some surrounding matter (such as the accretion disk corona[21]). Most of the models of the X2127+11/AC211 system have been based on this picture[2-4], but the type I X-ray burst from X2127+11 is inconsistent with this. The observed peak luminosity of the burst, $\sim 5 \times 10^{38}$ erg s^{-1}, is in excess of the Eddington limit for a 1.4-$M_{\odot}$ neutron star, which itself requires separate discussion[18]. The observed X-rays can hardly be a small fraction of the total burst emission, implying that the neutron star is directly visible. Consequently, the persistent luminosity, $\sim 6 \times 10^{36}$ erg s^{-1}, must be the unobscured source luminosity. This idea is consistent with the following observations, believed to be characteristic of a low-luminosity source[22,23]: (1) X2127+11 produces an X-ray burst and the burst is of long duration (≥ 150 s); (2) the energy spectrum of the persistent emission is a power-law type[18]. The lack of any X-ray eclipses is also consistent with the idea that we are directly viewing the central X-ray source[24]. This result indicates that a low $L_X/L_{\rm opt}$ does not necessarily mean that the source is screened from direct view. The optical luminosity, most of which is believed to come from reprocessing of X-rays, may be enhanced owing to an extended accretion disk corona, low metallicity of M15 and the low inclination of the binary system[25]. ☐

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Evidence from solar seismology against non-standard solar-core models

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GLOBAL oscillations of the Sun[1] have been used to test solar models[2], but modelling the oscillation frequencies to their measured accuracies of a few microhertz has proved difficult mostly owing to ignorance of the structure of the Sun's outer layers[3]. The frequency separation between closely spaced modes in the acoustic spectrum is expected to depend more on core properties[4], however, and thus to provide constraints on models of the solar core. Our observations combine data from a global network of observing stations, which reduces the masking effect of daily sidebands in the spectral analysis. Here we present precision measurements of fine structure and its variation with frequency. Our results agree with standard solar models[5-7], and seem to remove the need for significant mixing[8,9] or weakly interacting massive particles (WIMPS)[10,11] in the core, both of which have been advanced to explain the low measured flux of solar neutrinos[12,13]. This suggests that the solar neutrino problem must be resolved within neutrino physics, not solar physics; neutrino oscillations and a finite neutrino mass form a possible explanation.

Only three probes of the deep interior of the Sun are available to us at present—a possible gravitational quadrupole moment, solar neutrinos and solar seismology. The first of these could be due to rapid rotation or intense magnetic fields[14] and is a higher-order term of the gravitational potential, which could perturb the orbits of objects in the vicinity of the Sun, such as the planet Mercury or a future space probe. The excellent agreement between theoretical expectations of general relativity and observations of the rotation of the perihelion of Mercury and independent observational evidence of the validity of general relativity, from the deflections of radiowaves grazing the Sun[15] and the orbital evolution of binary pulsars[16], suggests that there is no substantial quadrupole moment. Independent evidence for the absence of large asymmetries is provided by the small visible oblateness of the solar surface[17] and by the small rotational splitting of the $l=1$ acoustic modes[18,19]. This combined evidence suggests that the assumption of a spherical Sun made in the standard model is an adequate approximation in the present context.

The second probe, solar neutrinos, have been detected in ^{37}Cl radiochemical observations[12] and, more recently, in neutrino-electron scattering observations[13]. The fact that detection rates are only about one-third of those predicted by standard solar models has become known as the solar neutrino problem[20]. Agreement between the two neutrino experiments, which use entirely different techniques, suggests that detection artefacts are not responsible for the problem. Explanations have been attempted both in terms of solar modelling and of neutrino properties. Among the former—essentially attempts to lower the core temperature and hence predicted neutrino production rates—have been higher hydrogen abundance owing to mixing[9], lower heavy-element abundance[21] and the possibility of WIMPS increasing the thermal conductivity[10,11]. The most plausible among the latter is the possible oscillation, resonantly enhanced in matter, between the three species of neutrinos—the Mikheyev-Smirnov-Wolfenstein (MSW) effect[22]. This would allow electron neutrinos produced in the solar core to be transformed into μ- and/or τ-neutrinos during their passage through the material of the Sun, thus reducing the count rate in the electron-neutrino detectors on Earth.

The third probe is solar seismology. With the benefit of hindsight, oscillations of small portions of the Sun were seen in the early 1960s[23], but probing of the deep interior only became possible when global oscillations were discovered and identified as acoustic eigenfrequencies eleven years ago[1]. These modes penetrate deeply into the interior and thereby probe temperature and mean molecular weight of the solar matter, and, more subtly, the rotation, magnetic fields and turbulence of the regions through which they pass. As emphasized in ref. 3, the contribution function is strongly weighted in favour of the surface layers (where the travel time is longest), but the precision of the measurement is so high that core contributions are well over two orders of magnitude greater than the observational errors.

Most models are unable to predict acoustic eigenfrequencies that agree with the data to better than a few microhertz (as compared with experimental uncertainties of substantially less than 1 μHz). The discrepancies between model predictions and the observed frequencies of acoustic modes are believed not to be fundamental but to be largely owing to the difficulties of modelling the outer layers of the Sun. It is possible to avoid these problems by measuring what is sometimes called the fine structure—the frequency separation δ_{ln}, where l is the degree and n is the order, between nearly coincident modes of l,n and $l+2$, $n-1$. Modes with low l all have similar behaviour in the outer layers (see, for example, ref. 4), but penetrate more deeply into the core as l decreases. This means that for low-l modes δ_{ln} are nearly independent of the outer layers but are sensitive to the structure of the core. Here we present results for δ_{0n} and δ_{1n} for a range of n.

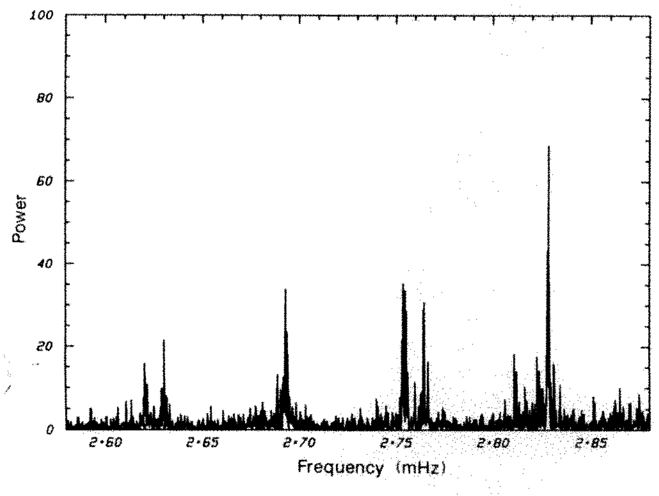

FIG. 1 Section of a typical power spectrum showing fine structure.

TABLE 1 Comparison of fine-structure predictions with observation

		δ_{0n}		δ_{1n}	
Reference	Label in Fig. 3	Intercept (μHz)	Slope (μHz)	Intercept (μHz)	Slope (μHz)
Data		9.00 (6)	−0.29 (3)	15.6 (1)	−0.46 (6)
5	A	9.38 (1)	−0.296 (4)	16.32 (1)	−0.415 (3)
6	B	9.14 (3)	−0.313 (7)	16.00 (3)	−0.441 (7)
6	C	9.03 (2)	−0.285 (7)	15.80 (1)	−0.410 (7)
7	D	8.77 (3)	−0.295 (7)	—	—
29	E	9.82 (8)	−0.29 (2)	17.1 (1)	−0.40 (2)
11	F	9.36 (8)	−0.426 (2)	15.30 (1)	−0.679 (4)
7	G	6.52 (3)	−0.486 (7)	—	—
7	H	7.88 (3)	−0.387 (7)	—	—
9	I	9.81 (3)	−0.28 (1)	—	—

Straight-line fit of splitting to $(n-21)$ over n=15–27. Models A to E are standard models. B and C have different opacities; G and H are models with more or less WIMPS. I is a mixed model. Numbers in parentheses are the 1σ errors in the last significant figure.

Until recently, measurements of acoustic (or p-mode) eigenfrequencies and hence of the fine structure have not been of sufficiently high accuracy to provide more than a crude test of theory. Our results enable us to quantify the variation with frequency.

Whole-disk measurements of the solar velocity have been carried out by the Birmingham Solar Seismology group since 1974 using the resonant-scattering technique[24]. Over the years a considerable amount of solar-velocity data has been accumulated. Here we consider results from measurements made in 1981–1988: combining data from several sites has enabled us to reduce the diurnal sidebands which severely contaminate p-mode fine structure (especially the $l=0$, $l=2$ difference) in single-site measurements. The data used here are a subset of those used in ref. 25, being two-month stretches of data from one, two or three stations, with a sampling interval of 42 s.

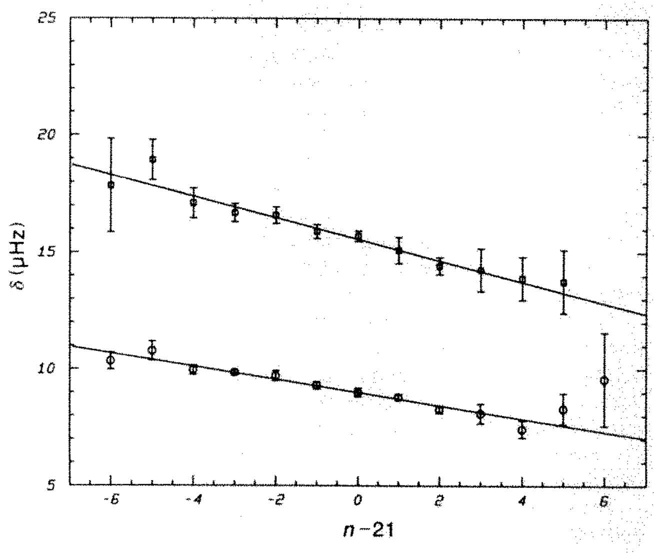

FIG. 2 Variation of the fine structure as function of $n-21$; the lower curve is δ_{0n} and the upper curve is δ_{1n}.

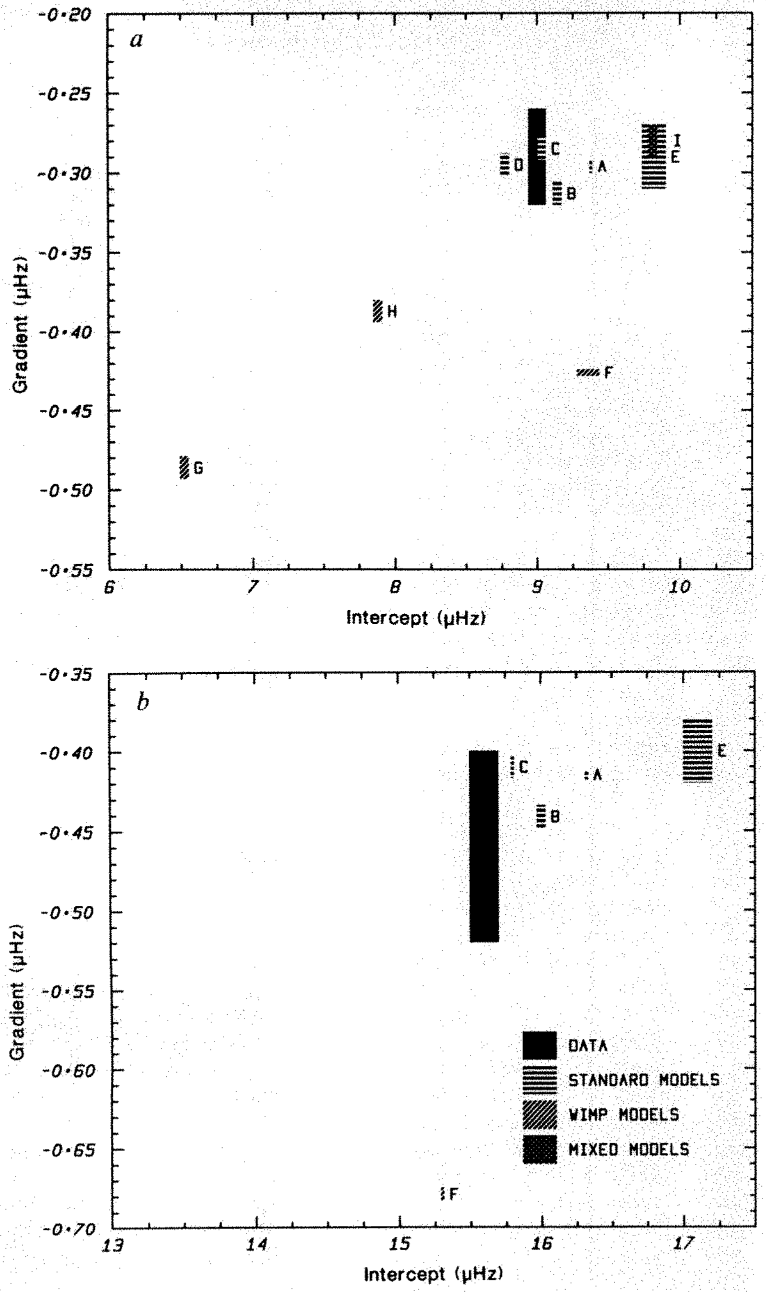

FIG. 3 Comparison of the observations with theoretical predictions. Both are parameterized by an intercept and a gradient of a linear fit. *a* refers to δ_{0n} and *b* refers to δ_{1n}.

A section of a typical power spectrum is shown in Fig. 1. The analysis was the same as that described in ref. 25.

Figure 2 shows the values of the frequency differences δ_{0n} and δ_{1n} as a function of n. The errors shown are the standard deviations of the means of up to eight values: not all modes could be measured in all spectra. In the range covered by the fitted values, these quantities show a significant decreasing trend with increasing n. The data may be fitted by a straight line; no significant improvement in the fits was obtained by using higher-order terms in n.

To test for temporal variation in the fine structure, a straight line was fitted to the values for individual years, in each case giving a gradient and intercept (with the $n-21$ axis) agreeing within errors with those of the mean of all years. Hence, although the individual frequencies vary over the solar cycle[25-28], we have no evidence of a significant time-dependence of the fine structure.

The gradients and intercepts from the fits are shown in Table 1 and Fig. 3, together with the predictions made by some theoretical models. These models include recent standard models[5-7], one mixed model[9] and WIMP models[7,29]. The gradients and intercepts for the models of Christensen-Dalsgaard, Cox *et al.*, Gough and Novotny were calculated from frequencies supplied by them, whereas the others were derived from fine-structure information published in graphical form in the papers referenced. Where no model information is shown for δ_{1n}, no comparable theoretical information is available.

Examination of Table 1 and Fig. 3 shows that, perhaps surprisingly, the agreement between observation and theory is best for recent standard models, the exception being the model of Bahcall and Ulrich[29]. Four years ago, theorists[30,31] were proposing that WIMP models gave the best fit to the fine structure. In the intervening years the models have changed (for example, by improvements in the equation of state and, above all, in the numerical precision of the integrations (J. Christensen-Dalsgaard, personal communication)) and so have their predictions in this context. By comparison our data, although of considerably better quality than previous observations[32], lie within the previous error bars.

Although further changes to solar models cannot be ruled out, the success of most current standard models in describing fine structure of the low-l acoustic modes may imply that the properties of the neutrino are responsible for the solar-neutrino problem. One possible explanation is provided by neutrino oscillations resonantly enhanced in their passage through matter —the MSW effect[22]. This implies neutrinos of finite mass and thus goes beyond the standard model of the electroweak theory[33]. □

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Light-emitting diodes based on conjugated polymers

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CONJUGATED polymers are organic semiconductors, the semiconducting behaviour being associated with the π molecular orbitals delocalized along the polymer chain. Their main advantage over non-polymeric organic semiconductors is the possibility of processing the polymer to form useful and robust structures. The response of the system to electronic excitation is nonlinear—the injection of an electron and a hole on the conjugated chain can lead to a self-localized excited state which can then decay radiatively, suggesting the possibility of using these materials in electroluminescent devices. We demonstrate here that poly(*p*-phenylene vinylene), prepared by way of a solution-processable precursor, can be used as the active element in a large-area light-emitting diode. The combination of good structural properties of this polymer, its ease of fabrication, and light emission in the green–yellow part of the spectrum with reasonably high efficiency, suggest that the polymer can be used for the development of large-area light-emitting displays.

There has been long-standing interest in the development of solid-state light-emitting devices. Efficient light generation is achieved in inorganic semiconductors with direct band gaps, such as GaAs, but these are not easily or economically used in large-area displays. For this, systems based on polycrystalline ZnS have been developed, although low efficiencies and poor reliability have prevented large-scale production. Because of the high photoluminescence quantum yields common in organic molecular semiconductors, there has long been interest in the possibility of light emission by these organic semiconductors through charge injection under a high applied field (electroluminescence)[1-7]. Light-emitting devices are fabricated by vacuum sublimation of the organic layers, and although the efficiencies and selection of colour of the emission are very good, there are in general problems associated with the long-term stability of the sublimed organic film against recrystallization and other structural changes.

One way to improve the structural stability of these organic layers is to move from molecular to macromolecular materials, and conjugated polymers are a good choice in that they can, in principle, provide both good charge transport and also high quantum efficiency for the luminescence. Much of the interest in conjugated polymers has been in their properties as conducting materials, usually achieved at high levels of chemical doping[8], and there has been comparatively little interest in their luminescence. One reason for this is that polyacetylene, the most widely studied of these materials, shows only very weak photoluminescence. But conjugated polymers that have larger semiconductor gaps, and that can be prepared in a sufficiently pure form to control non-radiative decay of excited states at defect sites, can show high quantum yields for photoluminescence. Among these, poly(*p*-phenylene vinylene) or PPV can be conveniently made into high-quality films and shows strong photoluminescence in a band centred near 2.2 eV, just below the threshold for π to π^* interband transitions[9,10].

We synthesized PPV (I) using a solution-processable precursor polymer (II), as shown in Fig. 1. This precursor polymer is conveniently prepared from α,α'-dichloro-*p*-xylene (III), through polymerization of the sulphonium salt intermediate (IV)[11-13]. We carried out the polymerization in a water/methanol mixture in the presence of base and, after termination, dialysed the reaction mixture against distilled water. The solvent was removed and the precursor polymer redissolved in methanol. We find that this is a good solvent for spin-coating thin films of the precursor polymer on suitable substrates. After thermal conversion (typically ≥ 250 °C, *in vacuo*, for 10 h), the films of PPV (typical thickness 100 nm) are homogeneous, dense and

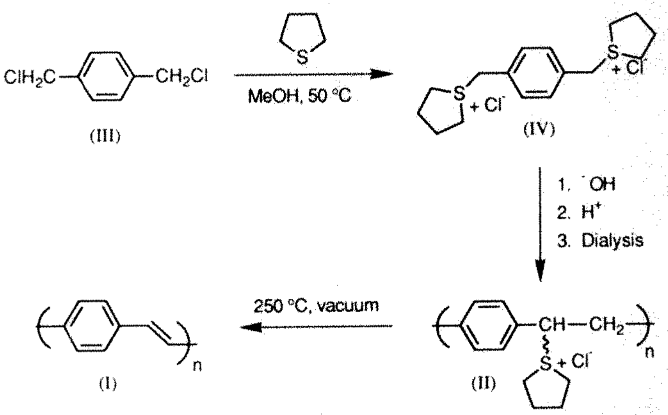

FIG. 1 Synthetic route to PPV.

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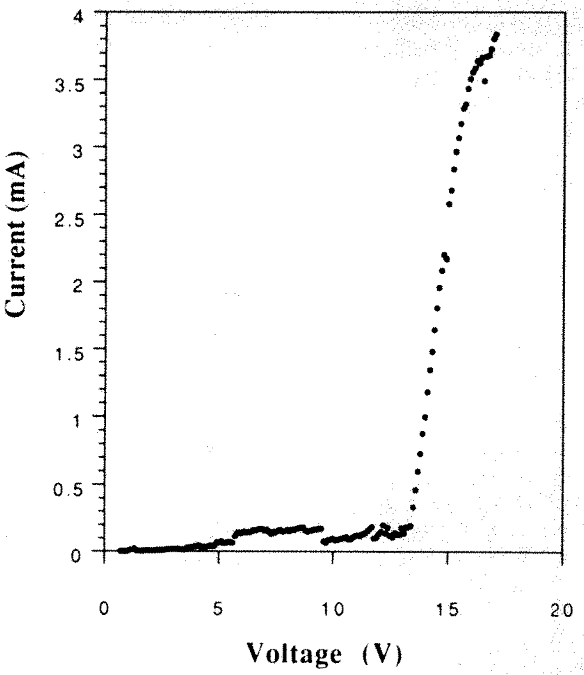

FIG. 2 Current–voltage characteristic for an electroluminescent device having a PPV film 70 nm thick and active area of 2 mm^2, a bottom contact of indium oxide, and a top contact of aluminium. The forward-bias regime is shown (indium oxide positive with respect to the aluminium electrode).

uniform. Furthermore, they are robust and intractable, stable in air at room temperature, and at temperatures >300 °C in a vacuum[11].

Structures for electroluminescence studies were fabricated with the PPV film formed on a bottom electrode deposited on a suitable substrate (such as glass), and with the top electrode formed onto the fully converted PPV film. For the negative, electron-injecting contact we use materials with a low work function, and for the positive, hole-injecting contact, we use materials with a high work function. At least one of these layers must be semi-transparent for light emission normal to the plane of the device, and for this we have used both indium oxide, deposited by ion-beam sputtering[14] and thin aluminium (typically 7–15 nm). We found that aluminium exposed to air to allow formation of a thin oxide coating, gold and indium oxide can all be used as the positive electrode material, and that aluminium, magnesium silver alloy and amorphous silicon hydrogen alloys prepared by radiofrequency sputtering are suitable as the negative electrode materials. The high stability of the PPV film allows easy deposition of the top contact layer, and we were able to form this contact using thermal evaporation for metals and ion-beam sputtering for indium oxide.

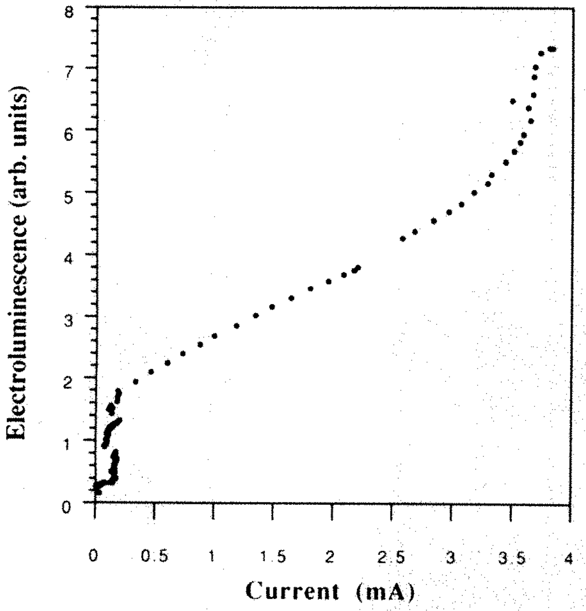

FIG. 3 Integrated light output plotted against current for the electroluminescent device giving the current–voltage characteristic in Fig. 2.

Figures 2 and 3 show typical characteristics for devices having indium oxide as the bottom contact and aluminium as the top contact. The threshold for substantial charge injection is just below 14 V, at a field of 2×10^6 V cm^{-1}, and the integrated light output is approximately linear with current. Figure 4 shows the spectrally resolved output for a device at various temperatures. The spectrum is very similar to that measured in photoluminescence, with a peak near 2.2 eV and well resolved phonon structure[9,10]. These devices therefore emit in the green-yellow part of the spectrum, and can be easily seen under normal laboratory lighting. The quantum efficiency (photons emitted per electron injected) is moderate, but not as high as reported for some of the structures made with molecular materials[2-7]. The quantum efficiences for our PPV devices were up to 0.05%. We found that the failure mode of these devices is usually associated with failure at the polymer/thin metal interface and is probably due to local Joule heating there.

The observation and characterization of electroluminescence in this conjugated polymer is of interest in the study of the fundamental excitations of this class of semiconductor. Here, the concept of self-localized charged or neutral excited states in the nonlinear response of the electronic system has been a useful one. For polymers with the symmetry of PPV, these excitations are polarons, either uncharged (as the polaron exciton) or charged (singly charged as the polaron, and doubly charged as the bipolaron)[15,16]. We have previously assigned the photoluminescence in this polymer to radiative recombination of the singlet polaron exciton formed by intrachain excitation[9,10] and, in view of the identical spectral emission here, we assign the electroluminescence to the radiative decay of the same excited state. The electroluminescence is generated by recombination of the electrons and holes injected from opposite sides of the structure, however, and we must consider what the charge carriers are. We have previously noted that bipolarons, the more stable of the charged excitations in photoexcitation and chemical doping studies, are very strongly self-localized, with movement of the associated pair of energy levels deep into the semiconductor gap, to within 1 eV of each other[9]. In contrast, the movement of these levels into the gap for the neutral polaron exciton, which one-electron models predict to be the same as for the bipolaron[15], is measured directly from the photoluminescence

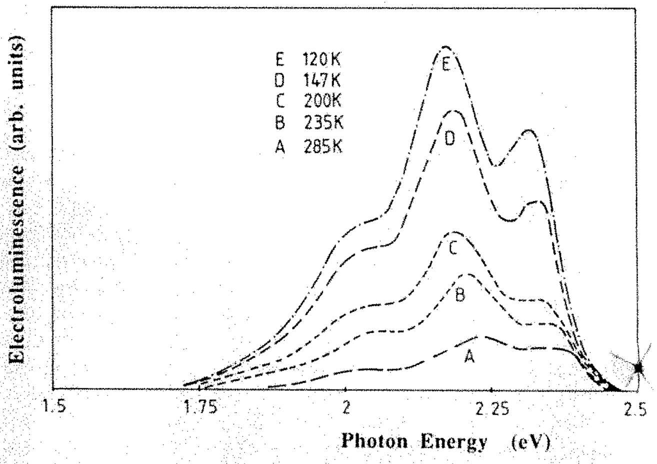

FIG. 4 Spectrally resolved output for an electroluminescent device at various temperatures.

emission to be much smaller, with the levels remaining more than 2.2 eV apart. For electroluminescence then, bipolarons are very unlikely to be the charge carriers responsible for formation of polaron excitons, because their creation requires coalescence of two charge carriers, their mobilities are low and the strong self-localization of the bipolaron evident in the positions of the gap states probably does not leave sufficient energy for radiative decay at the photon energies measured here. Therefore, the charge carriers involved are probably polarons. The evidence that they can combine to form polaron excitons requires that the polaron gap states move no further into the gap than those of the polaron exciton and may account for the failure to observe the optical transitions associated with the polaron.

The photoluminescence quantum yield of PPV has been estimated to be ~8%. It has been shown[10,17] that the non-radiative processes that limit the efficiency of radiative decay as measured in photoluminescence are due to migration of the excited states to defect sites which act as non-radiative recombination centres, and also, at high intensities, to collisions between pairs of excited states. These are processes that can, in principle, be controlled through design of the polymer, and therefore there are excellent possibilities for the development of this class of materials in a range of electroluminescence applications. □

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Tropospheric lifetimes of three compounds for possible replacement of CFC and halons

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CHLORINE and bromine have been implicated in the massive springtime depletion of stratospheric ozone over Antarctica[1]. As the source of these halogens is anthropogenic emissions of haloalkanes, the problem has acquired political and economic significance[2]. The chemical industry has been forced to consider urgently possible replacements for conventional halocarbons, and the potential effects on the environment of proposed alternative compounds have been evaluated recently in the Alternative Fluorocarbon Environmental Acceptability Study (AFEAS)[3]. Three such compounds that are not included in the AFEAS report are CF_2BrH (halon 1201). CF_3CFBrH (halon 2401) and $CF_3CF_2CCl_2H$ (HCFC 225ca). Removal of these compounds from the atmosphere will occur primarily by reaction with the hydroxyl radical, OH (ref. 4). Here we determine, from laboratory studies, the absolute rate of reaction between these three species and OH. Such kinetic data are vital for assessing their viability as replacement compounds. We use these data, along with our measurements of ultraviolet absorption cross-sections, to estimate the tropospheric lifetimes of the halons and HCFC 225ca against removal by OH, and their potential for destroying ozone in the stratosphere. Our approach shows how laboratory measurements can provide a useful first estimate of the environmental acceptability of compounds of this sort.

CF_2BrH and CF_3CFBrH are being considered as substitutes for CF_3Br in fire extinguishers, and $CF_3CF_2CCl_2H$ has useful properties as a solvent. The efficiency with which a unit mass of each halocarbon will destroy stratospheric ozone relative to the most important ozone-depleting molecules CFC-11 ($CFCl_3$) and CFC-12 (CF_2Cl_2) will depend strongly on the atmospheric lifetime of the compound. The daytime degradation of these hydrogen-containing molecules, RH, will occur primarily by reaction with the OH radical[4]

$$OH + RH \xrightarrow{k_1} H_2O + R \qquad (1)$$

and it is therefore important to know the rates of the homogeneous gas-phase reactions of OH with RH.

We have determined values of the second-order rate constants, k_1, for the hydrogen-atom abstraction process (1) and their variation with temperature for the three hydrohalocarbon molecules. Our experimental procedure and data analysis are described fully elsewhere[5-7]. We used an absolute discharge flow technique with a movable injection point for the reactant to provide time resolution; OH concentrations were measured by resonance fluorescence. The variation of OH concentration with injector position was monitored with a known excess of hydrohalocarbon added. The logarithm of the OH signal was a linear function of calculated contact time, and the gradient was plotted against the concentration of hydrohalocarbon to give a straight line of slope k_1. Our values for k_1 are given in Table 1 along with the experimental conditions. We used a conventional Arrhenius equation, $\ln k_1 = \ln A - E/RT$, to analyse the temperature-dependence. Thus a plot of $\ln k_1$ as a function of $1/T$ should give a straight line of slope E/R and intercept $\ln A$. Values of the activation energies E in the form E/R and pre-exponential factors A are given in Table 1. Errors on the slope, calculated in a linear least-squares analysis, are the 95% confidence limits. The 95% confidence limits on the intercept were unrealistically large, as is often the case in calculating Arrhenius parameters: we do not quote the errors here. The predictive capacity of the experimental Arrhenius expression is not properly represented by the individual errors on E and A, but rather by the combined expression incorporating both parameters.

We paid particular attention to the possible presence of fast-reacting impurities in the hydrohalocarbon samples, which could seriously affect the measurement of k_1. All of the

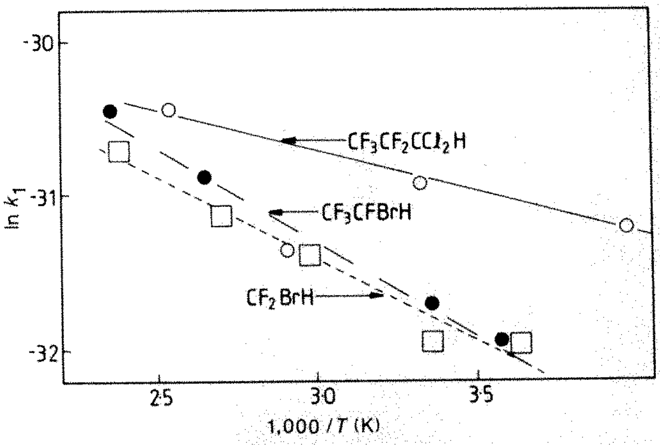

FIG. 1 Arrhenius plots for reactions of RH with OH.

TABLE 1 Kinetic data for reactions with OH

Temperature (K)	Number of runs	Flow-tube pressure (torr)	$10^{-11}[OH]_0$ (molecule cm^{-3})	$10^{-14}[RH]$ (molecule cm^{-3})	$10^{14}k_1$ (cm^3 molecule^{-1} s^{-1})	$10^{13}A$ (cm^3 molecule^{-1} s^{-1})	E/R (K)
CF_2BrH							
275	6	1.8–1.9	0.7	2.3–10.3	1.3±0.9	4.4	1,050±400
298	11	1.9–2.0	1.7–2.2	3.3–26.0	1.3±0.3		
336	6	1.9	0.7–0.9	3.1–26.8	2.3±0.3		
370	6	1.8–1.9	0.8	2.5–12.8	3.0±0.3		
420	6	1.8–1.9	1.0	2.2–10.9	4.6±0.9		
CF_3CFBrH							
279	7	1.8–1.9	0.8	4.8–24.0	1.3±0.4	11.3	1,250±350
298	12	1.9–4.7	0.9	4.0–59.1	1.7±0.3		
344	6	1.8	0.9	3.2–29.5	2.4±0.5		
377	6	1.8	0.7	1.8–13.6	3.9±0.5		
423	4	1.9	0.6	1.1–5.6	6.1±1.3		
$CF_3CF_2CCl_2H$							
251	6	1.8–1.9	0.9	2.1–10.0	2.8±1.3	2.3	550±750
300	11	1.8–1.9	0.7–0.9	1.8–12.3	3.7±0.8		
393	7	1.9	0.8–0.9	0.8–4.8	6.1±1.1		

compounds were obtained, with analyses, from ICI Chemicals and Polymers Limited and were used as received. The stated compositions were CF_2BrH 94.23% (major impurities: CF_2ClBr, 3.65%; CF_2HCl, 1.52%; CF_2Cl_2, 0.19%; $C_3F_6H_2$, 0.05%; CF_2HCF_2Cl, 0.4%; C_4F_6, 50 p.p.m.; C_3F_6, 10 p.p.m.; C_3F_5H, 10 p.p.m.), $CF_3CFBrH > 99.5\%$ (major impurities CF_3CFH_2, CF_2BrH and CF_2ClBr) and $CF_3CF_2CCl_2H > 99.5\%$ (major impurities HCFC-235 and HCFC-215); the main impurities in the compounds are other saturated halocarbons for which rates of reaction with OH are expected to be similar to those of the compound of interest. The contribution of the saturated species to the rate of loss of OH is thus negligible. Unsaturated impurities can potentially lead to more serious errors, because the addition of OH to an alkene double bond typically occurs at a rate 1,000 times larger than the abstraction of a hydrogen atom from a saturated halogen-containing alkane[8]. The worst case is for CF_2BrH, which has 0.005% of unsaturated impurity. If we assume a rate constant[8] of 10^{-11} cm^3 molecule^{-1} s^{-1} for the reaction between a halogenated alkene and OH, then 0.005% of alkene impurity would contribute 5% to the calculated second-order rate constant. The reactions between OH and the hydrohalocarbon molecules proceed by the abstraction process, reaction (1), and thus their rates are expected to be pressure-independent. We assume, therefore, that the kinetic data gathered in the laboratory at pressures of about 2 torr are also applicable to atmospheric conditions.

If we assume that reaction with OH is the only sink for hydrohalocarbons in the troposphere, then the tropospheric lifetime, τ, is $\{k_1[OH]\}^{-1}$. Scaling of $\tau_{CH_3CCl_3}$ by the ratio of the gas-phase rate constants at 277 K (ref. 9) for the reaction of OH with hydrohalocarbons and CH_3CCl_3 (methyl chloroform, MC)

$$\tau_{RH} = \tau_{MC} \times \frac{k_1(\text{MC, 277 K})}{k_1(\text{RH, 277 K})} \qquad (2)$$

as recommended by Prather[9], circumvents the need for reliable determination of the global mean concentration of OH, a parameter that is poorly established at present. τ_{MC} is better known

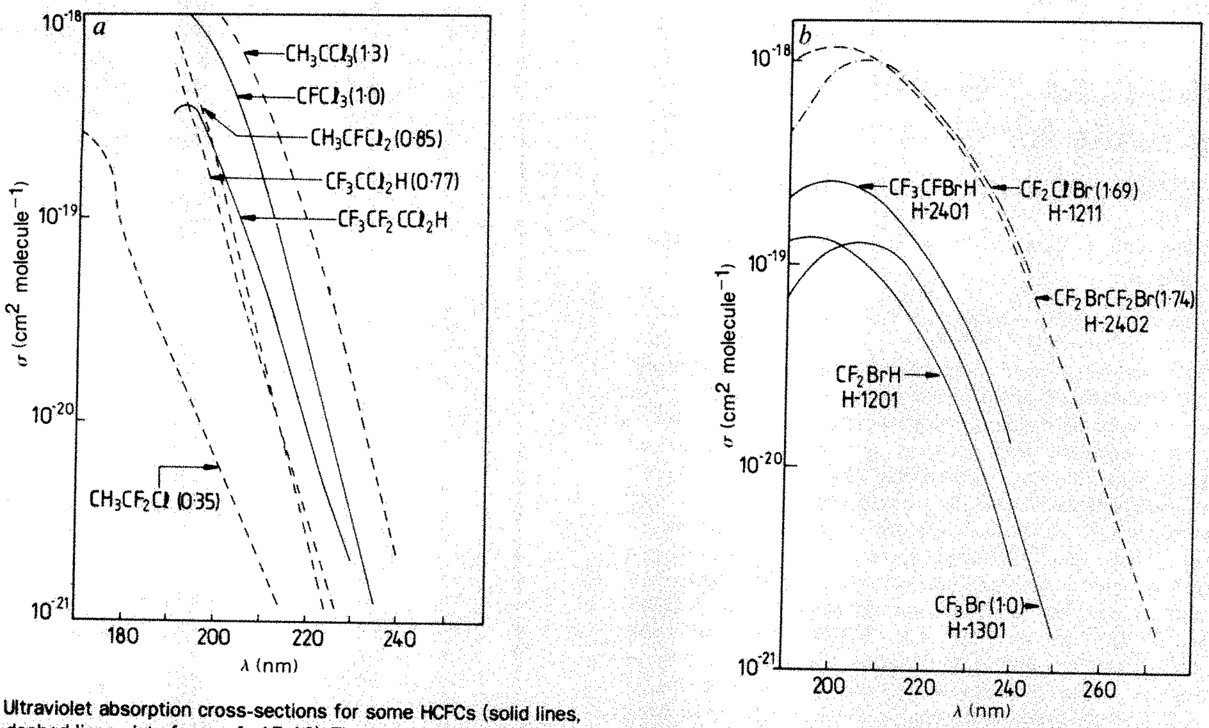

FIG. 2 *a*, Ultraviolet absorption cross-sections for some HCFCs (solid lines, our data; dashed lines, data from refs 15, 16). The numbers in parentheses are the CEFs taken from ref. 17. *b*, Ultraviolet absorption cross-sections for some brominated halocarbons (solid lines, our data; dashed lines, data from ref. 18). The numbers in parentheses are the BEFs taken from ref. 17.

TABLE 2 Chlorine- and bromine-loading potentials

Compound	10^{14} k_1 (277 K) (cm^3 molecule^{-1} s^{-1})	Lifetime (yr)	CLP or BLP
CF_2BrH	1.0	4.7*	0.07
CF_3CFBrH	1.2	3.9*	0.04
$CF_3CF_2CCl_2H$	3.2	1.5*	0.01
$CFCl_3$	—	60†	1.0
CF_3Br	—	72†	1.0
CH_3CCl_3	0.75	6.3†	0.11

* Lifetime calculated from equation (1).

† OSLO two-dimensional model results taken from ref. 14.

than the global concentration of OH because the atmospheric loading of CH_3CCl_3 has been established by field measurements and the only sources are anthropogenic, and documented[10]. Again, reaction with OH is assumed to be the only tropospheric sink for CH_3CCl_3, although Wine and Chameides[11] have suggested that hydrolysis of CH_3CCl_3 could contribute as much as 20% to its tropospheric loss. A value of 6.3 yr (ref. 12) for τ_{MC} was used in Prather's work and is the value used here. The estimated uncertainty in τ_{RH} when calculated in this way is approximately ±40%.

We use our values of τ_{RH} to determine the atmospheric chlorine-loading potential, CLP, as given by[13]

$$\mathrm{CLP(RH)} = \frac{\tau_{RH}}{\tau_{CFC\text{-}11}} \times \frac{M_{CFC\text{-}11}}{M_{RH}} \times \frac{n_{Cl}}{3} \qquad (3)$$

where $M_{CFC\text{-}11}$ and M_{RH} are the molecular weights of CFC-11 and RH, and n_{Cl} is the number of chlorine atoms in the hydrohalocarbon. We use an expression analogous to equation (3) to estimate a bromine-loading potential, BLP, relative to CF_3Br (halon-1301). The second most important factor influencing the potential of a halogenated compound to deplete stratospheric ozone is the dependence on altitude of the release of the active halogen (Cl and Br)[13], which in turn depends on the action spectrum for photolysis. For example, HCFC-142 has a CLP of 0.159 but an ozone depletion potential, ODP, of 0.05 (Oslo two-dimensional model[13]); the difference arises because HCFC-142 is photolysed in the upper stratosphere rather than lower down where there is a higher concentration of ozone. The ratio ODP/CLP (or ODP/BLP) is termed the chlorine (or bromine) efficiency factor, CEF (or BEF). We have measured the ultraviolet absorption spectra of the three compounds CF_2HBr, CF_3CFBrH and $CF_3CF_2CCl_2H$ using a commercial spectrophotometer (Perkin-Elmer Lambda 5). The spectra are compared with the spectra of similar substances in Fig. 2*a*, *b*. The absorptions of the three compounds are similar to those species for which the CEF or BEF are unity. The ODPs are therefore taken to be equal to the loading potentials for the compounds.

In Table 2, we list estimates of CLPs and BLPs based on our kinetic data. Although the loading potentials are lower for the possible CFC substitutes than for the CFCs themselves by virtue of their more rapid reactions with OH, another piece of information is required to determine the extent to which substitution of hydrohalocarbons for CFCs will lead to a reduction in the transport of chlorinated and brominated species to the stratosphere: to assess fully the implication for pollution of the troposphere and the stratosphere by the substitution of these compounds for CFCs, the fate of their oxidation products must be established. The possible degradation pathways of eight HCFCs and HFCs have recently been reviewed[14]. The main products of the oxidation of $CF_3CF_2CCl_2H$ are likely to be CF_3CF_2CClO and HCl. The degradation of bromine compounds was not considered in the review but products such as CF_2O from CF_2HBr and CF_3CFO from CF_3CFBrH are to be expected. Although the data gathered here are not sufficient for a rigorous evaluation of the ODPs (which requires detailed modelling), the combination of kinetic and spectroscopic data does provide a reasonable first estimate of the ODPs. ☐

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Influence of penetrating solar radiation on the heat budget of the equatorial Pacific Ocean

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SIGNIFICANT interannual variations in global climate, such as the El Niño/Southern Oscillation phenomenon (ENSO), result from interactions between ocean and atmosphere in the tropical Pacific. These interactions are mediated to a large degree by variations in the temperature of the sea surface, particularly in the warm, western portion of the basin[1–4]. Changes in sea surface temperatures (SSTs) provide precursors for the prediction of climate variability on ENSO timescales. But current ocean–atmosphere models, which estimate SSTs using climatological surface heat fluxes derived from ship observations[5–7], consistently overestimate SSTs in the western Pacific by as much as 3 K. Here we use recent satellite observations of ocean transparency, coupled with climatological surface heat fluxes and ocean density profiles, to show that solar radiation in visible frequencies, usually assumed to be absorbed at the sea surface, in fact penetrates to a significant degree to below the upper mixed layer of the ocean which interacts actively with the atmosphere. The net effect is a reduction of the heat input into the upper layer; for a 20-m-thick mixed layer this is equivalent to an annual reduction in temperature of about 5–10 K. Our results provide a natural explanation for the discrepancy between the SSTs predicted by models and those observed.

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The significance of the penetration of solar radiation to the heat budget of the oceanic mixed layer at low latitudes has been suggested theoretically[8-10] but only recently have the necessary synoptic observations of ocean transparency been available to estimate the magnitude of the associated heat flux and its significance to coupled ocean-atmosphere models of tropical SST. The NIMBUS-7 Coastal Zone Color Scanner (CZCS) made global observations of the colour of the surface ocean[11-13], which is directly related to its transparency to visible solar radiation[14]. We used an annual composite of CZCS observations of the equatorial Pacific during 1979 to determine the transparency of the ocean using empirical relationships between satellite-observed radiance from the water and spectral attenuation coefficients with a resolution of 10 nm over the bandwidth 350–700 nm[11,12,15]. These estimates were determined on a grid bounded by 10° N and 10° S, 120° E and 85° W with a spatial resolution of 5 °. Greater temporal resolution was not possible because of insufficient satellite coverage. Solar radiation flux at the sea surface was derived from climatological ship observations on the same grid[16] corrected for albedo and partitioned into the same spectral bands used in the determination of ocean transparency. Net surface heat fluxes, the sum of solar (including contributions from all wavelengths), latent, sensible and radiative terms, were also compiled from the same source. Mixed-layer depths, again with the same spatial resolution, were derived from climatological observations of vertical density distributions[17]; the depth of the mixed layer was defined as the depth at which the density had increased by 0.13 kg m^{-3} relative to the surface value. For each grid point, data were linearly interpolated between standard depths to estimate the mixed-layer depth. We used a density criteria for the mixed-layer depth, rather than the more traditional temperature criteria, because of the large influence of the salinity gradient on the density profile, particularly in the western Pacific[7,17,18].

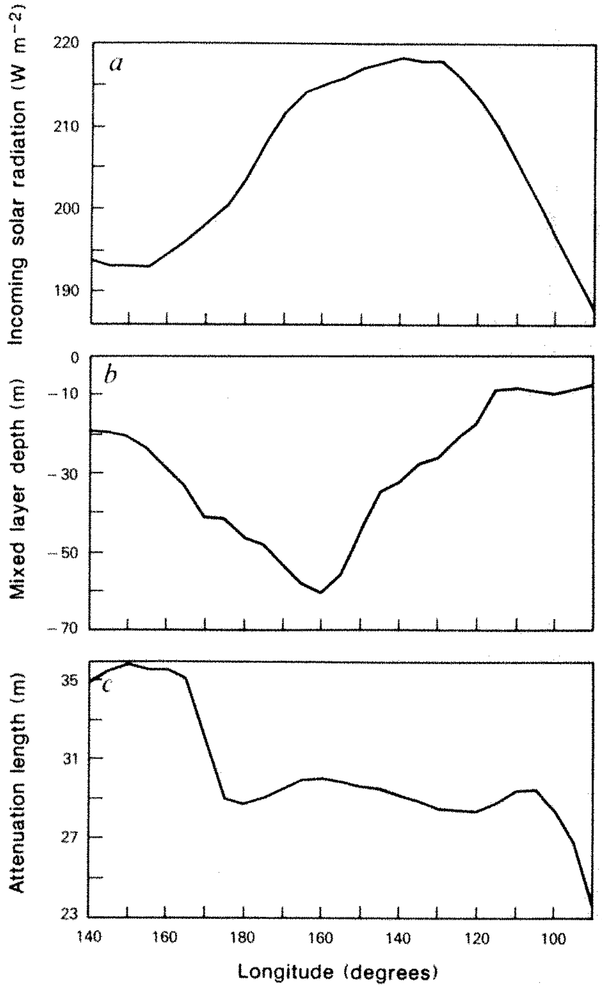

FIG. 1 Zonal patterns over the equatorial Pacific Ocean of *a*, solar radiation, *b*, mixed-layer depth and *c*, satellite-observed attenuation length scales ($1/K$) at 490 nm. Data have been meridionally averaged from 10° N to 10° S and zonally smoothed by a 15° running filter.

The penetration of surface radiation to the depth of the mixed layer for each wavelength band and at each grid point was calculated from

$$\bar{E}_{\mathrm{D}}(z_{\mathrm{m}}) = \int_{350}^{700} E_{\mathrm{D}}(\lambda, 0^-) \exp\left(-K(\lambda)\, z_{\mathrm{m}}\right) \mathrm{d}\lambda$$

where $\bar{E}_{\mathrm{D}}(z_{\mathrm{m}})$ is the solar irradiance penetrating to the depth of the mixed layer z_{m} (W m^{-2}), $E_{\mathrm{D}}(\lambda, 0^-)$ is the spectral irradiance just below the sea surface (W m^{-2} nm^{-1}), $K(\lambda)$ is the spectral attenuation coefficient (m^{-1}), and the integration is carried out over the visible portion of the spectrum. All calculations were carried out at full spatial resolution; for illustrative purposes, data were meridionally averaged.

Mixed layers based on the density difference of 0.13 kg m^{-3} are shallow (<25 m) over much of both western and eastern portions of the equatorial Pacific basin (Fig. 1*b*). To the east, most of the vertical density variation is due to a shallow thermocline; to the west, a shallow halocline is responsible for the depth of the mixed layer. Deeper mixed layers (>50 m) characterize most of the mid-basin region. This differs from the traditional view of a monotonically increasing mixed-layer depth from the east to the west[19], which is based solely on temperature distributions and does not reflect the strong influence of fresh water on the density structure in the western Pacific. The zonal pattern of solar radiation at the sea surface reflects the convective cloudiness associated with the Walker circulation; the highest radiation fluxes are in the region 150–120° W with lower fluxes to the east and west (Fig. 1*a*). The ocean transparency is extremely high to the west and is characteristic of pure sea water with little of the biogenic material, phytoplankton pigments and their breakdown products which discolour coastal regions (Fig. 1*c*). The transparency decreases to the east, where upwelling and a shallow nutricline bring nutrients to the sea surface, stimulating growth and development of higher concentrations of phytoplankton.

These patterns result in a clear zonal variation in the net flux of energy from the base of the surface mixed layer to the layer below (Fig. 2). Penetration rates are high (>25 W m^{-2}) in both east and west, and are lower in the mid-Pacific. Errors in the estimate of the net surface heat flux are dominated by systematic errors in the model bulk formulae[7,20] and are positively correlated with errors in the penetration term because both rely on an estimate of incoming solar radiation. Standard errors in the satellite estimate of the attenuation coefficient are small at these time and space scales (~10%) and contribute relatively little to the error in the magnitude of the penetration term. Given these sources of systematic and random error, we estimate that differences of < 15 W m^{-2} between the net surface heat fluxes and the flux associated with penetrating solar radiation are not likely to be significant. For most of the basin, the net flux of solar radiation downwards through the base of the mixed layer is equivalent to the climatological net surface heat flux into the ocean.

We conclude that for the equatorial Pacific Ocean, most of the net surface heat flux is lost to the mixed layer as solar radiation penetrates to heat the ocean below the depth of the layer that interacts most strongly with the atmosphere. One of the best defined precursors to six ENSO events has been an increase in SST of ~0.4 K over three months in the western Pacific[21]–the penetrating flux estimated here for the same region, 20 W m^{-2}, if absorbed in a 20-m layer, would result in a temperature increase of 1.8 K over a comparable time scale.

Current generations of equatorial coupled ocean–atmosphere

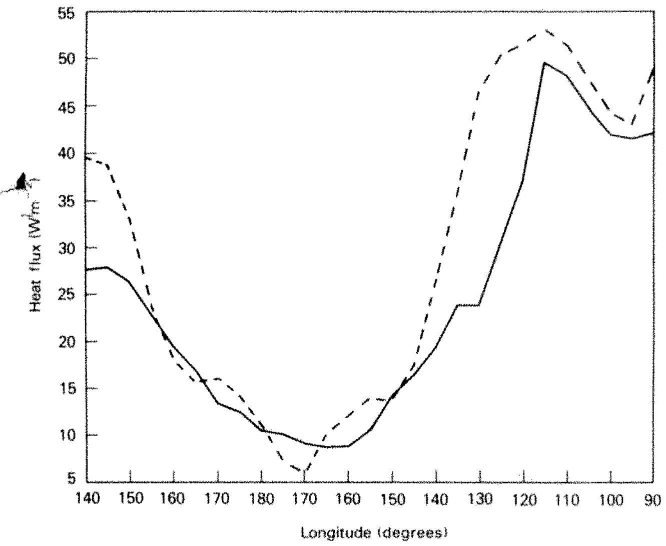

FIG. 2 Zonal patterns in the net flux of radiation from the base of the surface mixed layer (solid) and the net surface heat flux (dotted). Averaging as in Fig. 1.

models produce sea surface temperatures that are several degrees too high when climatological surface heat fluxes, derived from bulk formulae and ship-based meteorological observations, are used, suggesting that the estimates of positive net downward surface heat fluxes contain a systematic error and that the net heat flux into the ocean is close to zero over the western Pacific[5-7]. Some models of the Pacific warm-water pool[20] suggest that high rates of turbulent heat transport in the vertical are required to transport estimated net surface heat fluxes of the order of 20–30 W m^{-2} downwards out of the upper mixed layer. Although this turbulent flux may have a role in the region 150° to 115° W (Fig. 2) where there seems to be net heating of the mixed layer, these transports are not likely to be significant in the western Pacific where the shallow salinity 'barrier layer' prevents significant turbulent transport[7,19,21]. Other models have postulated an error in the formulae for heat flux, and have proposed corrections (increases in cloud attenuation) to force the model to match observed SSTs[6].

The analysis presented here permits a resolution of this apparent problem. The net radiative transport of heat downward through the base of the mixed layer is approximately equivalent to the estimated climatological net surface heat flux into the ocean over much of the western Pacific. The consequence is that the mixed layer receives effectively a negligible excess of heat over the year as required by the model SST evolution, even given a positive net downward surface heat flux.

One interesting consequence is the sensitivity of SST to variations in the optical properties of the upper ocean in the equatorial Pacific. For example, an increase in phytoplankton concentration in the western Pacific to the level typically observed in the east (0.3 mg chlorophyll m^{-3}), results in an increase in the heat trapped in the upper ocean of the order of 10 W m^{-2} owing to increased turbidity and absorption of solar radiation. Chlorophyll concentrations of this magnitude were observed in the western Pacific associated with the 1982/1983 ENSO event (Y. Dandonneau, personal communication).

The conclusions drawn here clearly indicate the necessity of considering the heat fluxes associated with penetrating solar irradiance in the development of predictions of tropical Pacific SST variability and its effect on the global climate. Future generations of ocean-observing satellites will be able to make extensive measurements of the surface heat fluxes[22,23] and the optical transparency[24] of the tropical ocean and thus provide an input to future coupled ocean-atmosphere models. □

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Extreme temporal homogeneity of helium isotopes at Piton de la Fournaise, Réunion Island

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OCEAN island basalts (OIBs) have strontium, neodymium and lead isotopic compositions that are different from those of mid-ocean-ridge basalts, (MORBs), reflecting long-term differences in the chemical characteristics of the respective mantle source reservoirs. The high $^{3}\text{He}/^{4}\text{He}$ ratios at some islands such as Hawaii and Iceland[1-7] indicate that these basalts come from sources that are less degassed than the source of MORB. Many islands exhibit considerable variability in Sr, Nd and Pb isotopes[8-10], but detailed studies of temporal variations in helium isotopes have been restricted to Hawaiian volcanoes—at Mauna Loa, for example, significant variations in $^{3}\text{He}/^{4}\text{He}$ have been found for the past 30,000 years[11]. Here we report on $^{3}\text{He}/^{4}\text{He}$ ratios from Piton de la Fournaise volcano on Réunion Island. No variations are found over the long time of 360,000 years, indicating a remarkable uniformity of $^{3}\text{He}/^{4}\text{He}$ for the (large) mantle source region over this timescale. The He–Sr–Pb systematics at this island may reflect the simultaneous contribution of both recycled materials (perhaps subducted crust) and primitive components to the Réunion source.

Réunion Island (21° 7′ S, 55° 32′ E) is the present location of the hotspot trace that trends northeast through Mauritius Island, along the Mascarene Plateau toward the Deccan Traps in India (Fig. 1)[12]. The total distance of ~5,000 km makes this hotspot chain comparable in length to the Hawaiian Island chain. Such hotspot traces are believed to result from the interaction of a relatively fixed, hot rising plume in the Earth's mantle with the moving lithosphere[13]. Réunion Island is located within the Dupal anomaly, which is a broad, nearly globe-encircling band centred around 30° S[14,15]. Many islands in this band, including

Réunion[16-19], have characteristically high $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$ and $^{87}Sr/^{86}Sr$ ratios, reflecting ancient chemical fractionations of U/Pb, Th/U and Rb/Sr. Dupal islands are sometimes thought to contain subducted crust or lithosphere as a mantle component[20-23], or to be produced by ancient mantle metasomatism[23,24].

Réunion Island has two volcanoes; the extinct volcano Piton des Neiges comprises about two-thirds of the island to the northwest, and the presently active Piton de la Fournaise is located in the southeast. Piton des Neiges forms the basement on which Piton de la Fournaise began to grow sometime before ~350,000 yr ago[25]. The Piton de la Fournaise eruptions are well documented both historically and chemically and consist of picrites and lavas that are transitional between alkali basalts and tholeiites[18,19,26,27]. We have measured $^3He/^4He$ and $^{87}Sr/^{86}Sr$ ratios in historic and prehistoric lavas from Piton de la Fournaise. Helium trapped in inclusions was extracted by *in vacuo* crushing of olivine phenocrysts separated by hand-picking under a microscope. Recent lavas studied come from historical eruptions in 1708, 1776 and 1800 AD, and eight others since 1931, including the picritic eruptions of 1931, 1961 and 1977. Prehistoric lavas studied include a basalt dated by ^{14}C on charcoal (455 AD), four basalts dated by U-Th disequilibrium (<7,000, 15,000, 97,000 and 106,000 years old) and two picrites dated by K-Ar (327,000 and 360,000 years old)[25].

He isotopes were measured on a mass spectrometer designed and built at the University of California (Santa Barbara) by J. L. The instrument is a 90° curvature, 21-cm radius, statically operated, double-collector mass spectrometer. The sensitivity for He is $>10^{-4}$ A torr^{-1}, and the absolute detection limit is $<10^4$ atoms of ^3He. The inlet system uses a low-temperature (40 K) charcoal trap for separation of He from other rare gases. The precision for He isotopic ratio determinations is very close to the limit based on ion counting statistics for the weak ^3He beam. The total system blank is $\sim 1\times10^{-10}$ cm^3 STP ^4He. Blanks were always run before samples. A secondary standard of Yellowstone Park gas was always run after samples, with standard size similar to the size of the sample just analysed. This gas has been routinely calibrated against marine air at UCSB and has a $^3He/^4He$ ratio of 16.49 ± 0.04 (2σ) R_A (R_A is the atmospheric ratio of 1.39×10^{-6})[28]. This procedure allows very small samples to be analysed and a precise check of sample isotopic ratios in the size range of the analysis.

Our He results (Table 1) confirm the earlier observations that Réunion Island is a high $^3He/^4He$ hotspot[29-33]. In addition, the temporal and spatial homogeneity of $^3He/^4He$ is remarkable. There has been no discernible change on any timescale between ~1 year and 360,000 years at Piton de la Fournaise (Fig. 2). The He isotope compositions of the picrites are indistinguishable from the basalts. The mean $^3He/^4He$ ratio for all samples is $\sim 12.9\pm0.4$ (2σ) R_A; that is, the variation in $^3He/^4He$ is only 3% ($n=20$). This is in marked contrast to the temporal variability in $^3He/^4He$ found at Hawaiian volcanoes[11].

The constant He isotopic ratios contrast with the petrological and trace-element data, which indicate that the historical picrites are produced by disruption of pre-existing, genetically unrelated olivine-rich cumulates, apparently by pulses of basaltic magmas on a timescale of about 17 years[19]. Also, the formation of Dolomieu caldera in 1930, which represented a large change in magma composition[19], was not accompanied by a change in $^3He/^4He$. The minor variability in He and Sr isotopic ratios ($^{87}Sr/^{86}Sr = 0.70406$-$0.70425$; Table 1) indicates that the source region has remained homogeneous during the entire sub-aerial history, and that melting conditions have remained uniform. We can estimate the scale of source homogeneity by assuming that the volumetric production rate of 0.08 km^3 yr^{-1} since 1931[26] is grossly representative of the sub-aerial history, and that 7% partial melting of the mantle source is applicable[19]. The characteristic length scale for He isotope homogeneity in the mantle source is then $\sim(0.08\times360{,}000/0.07)^{1/3}$, or >70 km. Constant $^3He/^4He$ ratios also suggest short transport times of magma from the source region ($<10^4$ years) and the absence of a long-lived storage reservoir at depth, consistent with inferences based on U-Th disequilibria[34]. The homogeneity of Sr isotopes is circumstantial evidence that the homogeneity of helium is not due to domination by metasomatic fluids, and also makes it unlikely that external components from the asthenosphere or lithosphere have been added on the way to the surface. Helium is therefore a genuine tracer that is transferred on melting from the source rock to the melt. The $^3He/^4He$ record at Piton de la Fournaise is best explained as the result of a well mixed source region, rather than as a mixture of distinct fluid and/or magmatic

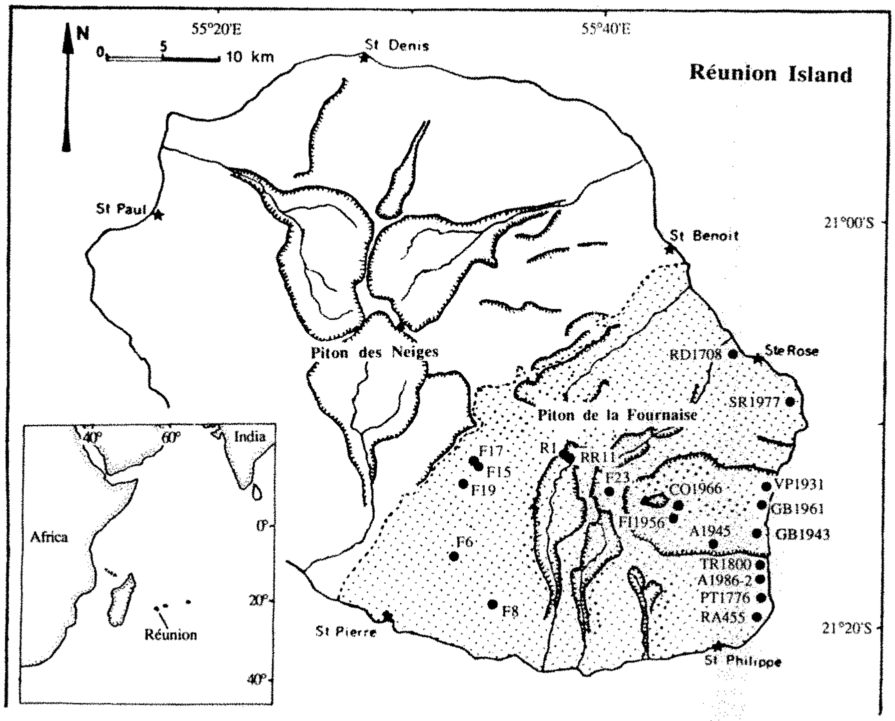

FIG. 1 Sample locations from Piton de la Fournaise, Réunion Island. Inset shows the island location in the Indian Ocean (after ref. 26).

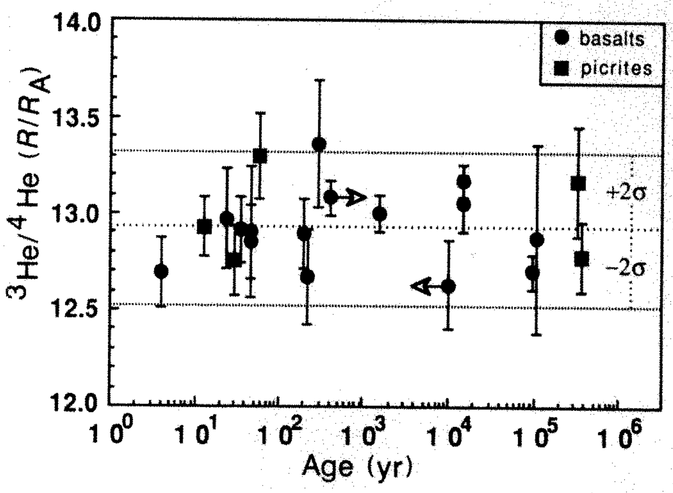

FIG. 2 ^{3}He/^{4}He plotted against age for basalts and picrites from Piton de la Fournaise. The two samples with arrows are plotted at their age limits (a prehistoric flow and a sample which is <7,000 yr old). Where replicate analyses were performed the average result is shown. The dashed lines show the mean value of all the data $\pm 2\sigma$. Error bars for individual samples are $\pm 1\sigma_{\text{mean}}$.

components, which we would expect to show temporal variability.

^{3}He/^{4}He ratios along mid-ocean ridges are typically between 7-9 R_A. The relatively high ^{3}He/^{4}He ratios at Réunion compared to MORB may indicate some contribution from relatively undegassed mantle, similar to that beneath other hotspots such as Hawaii and Iceland. Réunion is, however, located within the Dupal anomaly. ^{3}He/^{4}He ratios lower than MORB values are found at Dupal islands such as Tristan da Cunha and Gough[2], consistent with a higher (U+Th)/^{3}He produced by crustal recycling or by mantle enrichment events. In contrast, the ^{3}He/^{4}He results for Piton de la Fournaise indicate that some Dupal islands can also have 'primitive' He isotope signatures. Castillo[35] showed that Dupal anomaly maxima are correlated with low seismic velocities in the lower mantle and with the locations of hotspots. If the relatively high ^{3}He/^{4}He ratios at hotspots such as Hawaii, Iceland and Réunion are evidence for some degree of chemical isolation or layering in the mantle[36-39], then the He-Sr-Pb isotope systematics at Réunion also suggest that recycled materials (perhaps slabs) may penetrate into lower-mantle regions, where they may acquire relatively primitive rare-gas signatures but retain much of their enriched Sr and Pb

TABLE 1 He and Sr isotopes in basalts and picrites from Piton de la Fournaise

Sample	Age (years)	Weight (mg)	[He] (10^{-9} cm^3 STP g^{-1})	^{3}He/^{4}He (R/R_A)	$2\sigma_{\text{mean}}$	$\frac{\text{He/Ne}}{\text{He/Ne}_{\text{air}}}$	^{87}Sr/^{86}Sr
Historical samples							
RD1708	282	197.7	4.17	13.36	0.66	18.3	0.70424
PT1776	214	451.3	3.91	12.67	0.48	35.4	0.70419
TR1800	190	210.8	9.37	12.89	0.36	48.0	0.70410
VP1931†	59	363.1	3.28	13.64	0.62	20.1	0.70413
replicate		611.1	6.17	12.96	0.28	65.6	
GB1943	47	135.4	7.06	12.90	0.68	19.2	0.70410
A1945	45	307.6	8.14	12.85	0.38	53.2	0.70413
FI1956	34	174.3	18.1	12.92	0.34	67.6	0.70424
GB1961†	29	496.7	3.78	12.82	0.42	29.8	0.70421
replicate		430.2	8.46	12.69	0.30	74.9	
CO1966	24	179.3	7.96	12.97	0.52	36.5	0.70419
SR1977†	13	464.8	5.41	12.80	0.40	46.8	0.70424
replicate		442.9	15.5	13.06	0.22	120	
A1986-2	4	133.0	13.2	12.69	0.36	51.1	0.70423
Dated samples							
F6*†	360,000 ± 10,000	349.4	3.52	13.17	0.58	27.2	0.70415
F8*†	327,000 ± 20,000	360.0	8.37	12.78	0.36	54.4	0.70422
R1	106,000 ± 16,000	417.0	1.58	12.87	0.99	7.6	0.70406
RR11	97,000 ± 14,000	463.3	32.2	12.70	0.17	243	0.70425
F15	~15,000	419.1	11.2	13.05	0.30	73.1	
F17	15,000 ± 5,000	263.0	46.8	13.17	0.18	236	0.70425
F19	<7,000	178.4	9.08	12.63	0.46	34.4	0.70425
F23	prehistoric	237.0	78.0	13.08	0.17	383	0.70426
RA455	1,535	284.0	30.9	13.00	0.20	173	0.70416

Historical sample identification numbers refer to year (AD) of eruption. Sample localities for historic eruptions are described in ref. 19. F6 and F8 are from near le Tampon, R1 and RR11 from near Nez de Boeuf, F15, F17 and F19 from Plaine des Cafres (Piton Hyacinthe) and F23 from Piton Chisny (Fig. 1). All He results are for olivine mineral separates. Reproducibility of air-standard He sizes is better than 3% (2σ); sample He contents vary much more owing to the variability in abundance of inclusions. All Ne contents were less than twice the blank value and He/Ne ratios should therefore be considered minimum values. Sr isotope results are for whole rocks ($2\sigma < 0.00004$). Sr isotope analyses of historical samples and RA455 were performed at the Centre de Recherches Petrographiques et Geochimiques, some of which were previously reported[19]; Sr analyses of dated samples were performed at the Centre de Recherches Volcanologiques. U-Th ages are from Condomines and Bachelery (manuscript in preparation).

* K-Ar ages[25].

† Picrite.

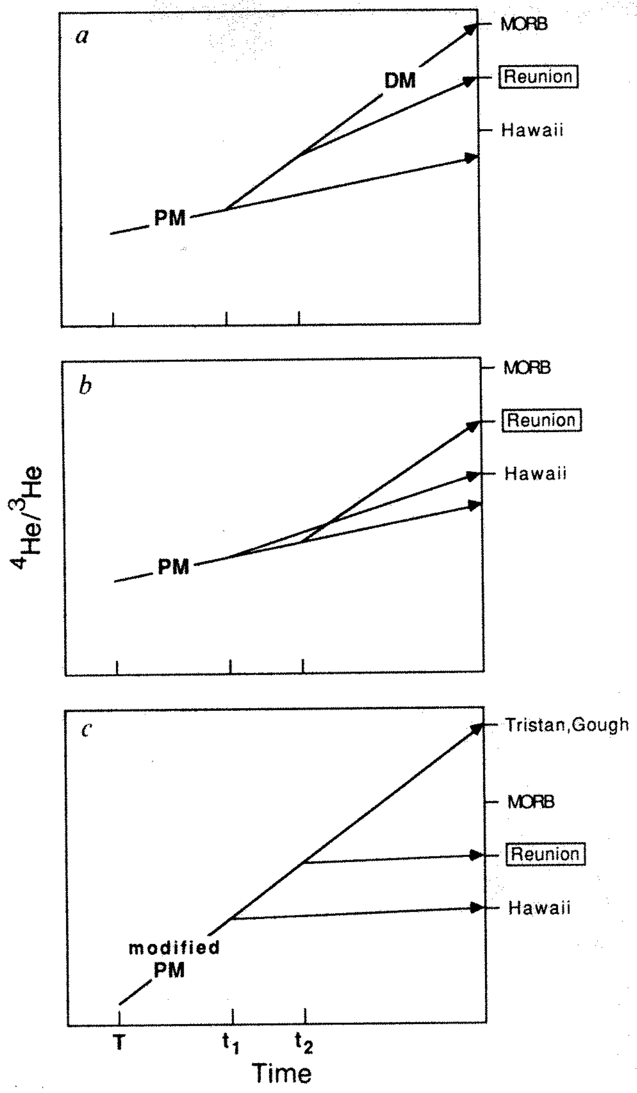

FIG. 3 Simplified evolutionary models for $^4He/^3He$ in the Réunion mantle source. Lines shown are schematic because of the nonlinear production rate of ^4He by radioactive decay of ^{238}U, ^{235}U and ^{232}Th. T is the age of the Earth. Relative He isotope compositions for OIBs and MORBs are shown on the right. Melt extraction always leaves a residue with lower Rb/Sr and Th/U. In *a* and *b*, He is assumed to be more incompatible than U and Th during partial melting; in *c* He is less incompatible than U and Th. *a*, Model 1—Réunion source formed at t_2, by metasomatic enrichment of depleted mantle (DM) with fluids having high Rb/Sr, Th/U and low $(U+Th)/^3He$. DM was formed as a residue of melt extraction from primitive mantle (PM) at t_1. Radiogenic Sr and Pb isotopes at Réunion do not allow the source to be a residue of melt extraction from DM, and the Sr and Nd isotopes in Hawaiian basalts with high $^3He/^4He$ are not representative of PM. *b*, Model 2—Réunion source formed as a residue of melt extraction from PM. This decreased Rb/Sr and Th/U, but increased $(U+Th)/^3He$ relative to PM. The case shown assumes similar Rb/Sr and Th/U fractionations at t_1 and t_2; because the Hawaiian source has lower $^{87}Sr/^{86}Sr$ and $^{208}Pb/^{204}Pb$ it would be 'older' than the Réunion source in this case. The case of larger Rb/Sr and Th/U fractionations at t_2 compared to t_1 is also possible, in which case the Réunion source would be formed at t_1 and the Hawaiian source at t_2. In either case the fractionations in $(U+Th)/^3He$ and Rb/Sr are not strictly correlated, because Hawaii has higher $^3He/^4He$ and lower $^{87}Sr/^{86}Sr$ than Réunion. *c*, Model 3—Réunion source formed as a residue of melt extraction from 'modified PM' at t_2. Early outgassing produced PM with high $(U+Th)/^3He$. Subsequent melt extraction produced a residue with lower Rb/Sr, Th/U and $(U+Th)/^3He$. The isotope signature for PM is that of bulk Earth ($^{87}Sr/^{86}Sr = 0.7047$ and $^3He/^4He = 5\ R_A$). As DM also lost U and Th throughout its history whereas PM did not, DM evolved to higher $^3He/^4He$ than 'PM', consistent with MORB values of 7–9 R_A. Parent/daughter fractionations are all in the same direction; the magnitudes of the fractionations are also correlated between the Hawaiian and Réunion sources, but not between DM and OIB sources, as DM has the lowest $^{87}Sr/^{86}Sr$ but intermediate $^3He/^4He$.

isotope signatures. In this case, both primitive and recycled components may contribute simultaneously to the genesis of an individual island such as Réunion.

In addition to the complex mixing described above, other possible explanations for the He-Sr-Pb relations at Réunion include: (1) old enrichment of depleted mantle by fluids having high Rb/Sr, U/Pb, Th/U and low $(U+Th)/^3He$; (2) ancient differentiation from primitive mantle by partial melt extraction; (3) ancient differentiation from a remnant of primitive mantle that was outgassed during an early stage of Earth history. These alternatives are qualitatively outlined in Fig. 3. Model 3 leads to the idea that helium may not be as effectively outgassed as is often assumed. In the absence of fluids, such as in the deep mantle, helium is simply partitioned into the melt phase as an incompatible element. Depending on the mineralogical constitution of the source, He might sometimes be more compatible than U or Th because He solubility in solids is not much less than in melts[40,41]. Under these circumstances partial melting would leave a residue with lower $(U+Th)/^3He$, and with time promote increasingly higher $^3He/^4He$ ratios in the residual peridotite (harzburgite) compared to more fertile sources. Because melt extraction should also leave a residue with low Rb/Sr and Th/U, the lower $^{87}Sr/^{86}Sr$ and $^{208}Pb/^{204}Pb$ and higher $^3He/^4He$ ratios at islands such as Hawaii and Iceland could suggest that their mantle sources are residues of earlier differentiation of the same reservoir which later produced the Réunion source.

Collectively, the uniform and relatively high $^3He/^4He$ ratios coupled with moderately radiogenic Sr and Pb isotopes at Réunion Island can be reconciled with either a simultaneous contribution of recycled and primitive components to the genesis of an individual ocean island volcano, or with metasomatism or ancient differentiation that in some cases produced a decrease in $(U+Th)/^3He$ in the mantle. ☐

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A new biogeographic connection between islands in the Atlantic and Pacific Oceans

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THE affinities between floras of widely separated regions of the world, as well as modes of dispersal of elements in these floras, have long been of interest in evolutionary and phytogeographical studies[1-3]. While investigating phylogenetic patterns and modes of speciation in the endemic plants of the Juan Fernandez Islands, Chile[4-6], we have documented an inter-population separation (disjunction) for the plant genus *Peperomia* (Piperaceae) of more than 5,000 km, one of the longest known in flowering plants. *Peperomia berteroana* ssp. *berteroana* occurs only in the Juan Fernandez Islands in the Pacific Ocean, and *P. berteroana* ssp. *tristanensis* is restricted to the Tristan da Cunha archipelago in the South Atlantic Ocean. Flavonoid and morphological data suggest that plants from Masafuera, the younger of the Juan Fernandez Islands, are ancestral to Tristan da Cunha populations. We propose that long-distance dispersal by birds is the most likely cause of this wide disjunction.

The Juan Fernandez Islands are located 600 km west of continental Chile at about 33° S, 80° W (Fig. 1). The two principal volcanic islands are Masatierra (MT), which is roughly 4 Myr old, and Masafuera (MF), which is 150 km farther west, and 1-2 Myr old[6]. Four distinctive species of *Peperomia* occur in this archipelago: *P. fernandeziana* Miq. (Chilean mainland, MT, MF), *P. margaritifera* Bert. ex Hook. (MT), *P. skottsbergii* C.DC. (MF) and *P. berteroana* Miq. (MT, MF). Comparative morphological studies reveal that the latter species is nearly identical to *P. tristanensis* Christoph., which was treated as a synonym of *P. berteroana* by Christophersen[7] and Groves[8] and more recently as two distinct subspecies[9]. *Peperomia berteroana* ssp. *tristanensis* occurs in Inaccessible Island of the Archipelago of Tristan da Cunha, which is in the middle of the South Atlantic Ocean (37° S, 12° W) (Fig. 1). This archipelago is relatively young[10,11] and consists of three islands: Tristan (0.5 Myr), Inaccessible (2.9 Myr) and Nightingale (18 Myr).

Observations and collections for morphological and chemical studies were made on *Peperomia berteroana* and relatives during three field expeditions to the Juan Fernandez Islands (1980, 1984, 1986). Existing herbarium material was also examined. Twenty-seven morphological characters, including both vegetative and reproductive features, were analysed using numerical taxonomic techniques[12]. Leaf-shape, number of principal leaf veins, blade vestiture and spike arrangement showed the most notable discontinuities.

Principal components analysis (Fig. 2) of morphological features reveals *Peperomia berteroana* ssp. *tristanensis* as close to, but clearly distinct from, ssp. *berteroana*. However, of all the populations of ssp. *berteroana* in the Juan Fernandez Islands, some of those from Masafuera are closest morphologically to ssp. *tristanensis* (Fig. 2).

Subspecies were compared for flavonoid compounds sequestered in their leaves. A total of 15 flavones, 5 with sulphate groups, were isolated and identified through high-voltage paper electrophoresis and two-dimensional paper chromatography (Table 1). There is a pattern of island-specific, rather than taxon- or archipelago-specific flavone profiles. The most notable flavonoid differentiation is seen between plants on Masatierra and those of the Masafuera–Inaccessible island group. Even though the latter two islands belong to two archipelagos separated by more than 5,000 km, plants occuring in them have four compounds in common, none of which is present in the Masatierra populations.

TABLE 1 Occurrence of flavones in subspecies of *Peperomia berteroana*

	Flavones													
Subspecies	1	2	3	4	5	6	7	8	9	10	11	12	13	14
berteroana (MT)	+	+	+	+	+	+	+	+	+	+				
berteroana (MF)	+	+	+	+	+	+	+				+	+	+	+
tristanensis	+	+	+	+	+	+	+				+	+	+	+

1, Acacetin; 2, Ac-7-sulphate; 3, Di-7-*O*-monoglucoside; 4, Di-7-*O*-rhamnoglucoside; 5, Lu-7-*O*-monoglucoside; 6, Lu-7-*O*-diarabinoside; 7, Lu-7-sulphate; 8, Ap-7-glucosidesulphate; 9, Lu-7-*O*-rhamnoglucoside; 10, Di-7-*O*-glucoside; 11, Ac-7-glucosidedisulphate; 12, Di-*C*-arabinoglucoside; 13, Di-7-*O*-arabinoglucoside; 14, Di-7-sulphate. (Ap, apigenin; Ac, acacetin; Di, diosmetin; Lu, luteolin.)

This particular disjunct distribution could be explained by: (1) parallel evolution from a common ancestor in South America; (2) convergent evolution from different ancestors; (3) continental drift; or (4) long-distance dispersal by birds.

The first suggests that the two subspecies would have evolved in parallel from a common ancestral population and would have spread from South America to both archipelagos by means of bird dispersal within the past 3 Myr. This time-frame is suggested by the known ages of the islands where *Peperomia* now grows, and by the slight morphological and chemical differentiation between the Juan Fernandez and Tristan da Cunha populations.

No linking populations, or a common ancestral population with the characteristics shown by the taxa on both islands, have been discovered in South America, despite extensive searches in different herbaria rich in collections of *Peperomia*. Therefore, the ancestor either does not exist in South America, has become extinct (fossils of *Peperomia* are not known from these antecedent localities), or it has not so far been discovered.

The position of the Tristan da Cunha archipelago on the Mid-Atlantic Ridge suggests that there could have been numerous islands that may have served as stepping stones between South America and Africa during the gradual opening of the Atlantic. However, the poverty and general composition of the Tristan flora, as compared with that of South America and Africa, do not support this possibility. Prominent members of the fern floras of both South America and Africa are absent[13]. Migration across archipelagos would be expected to result in a richer flora than the islands have, and one that contains a better

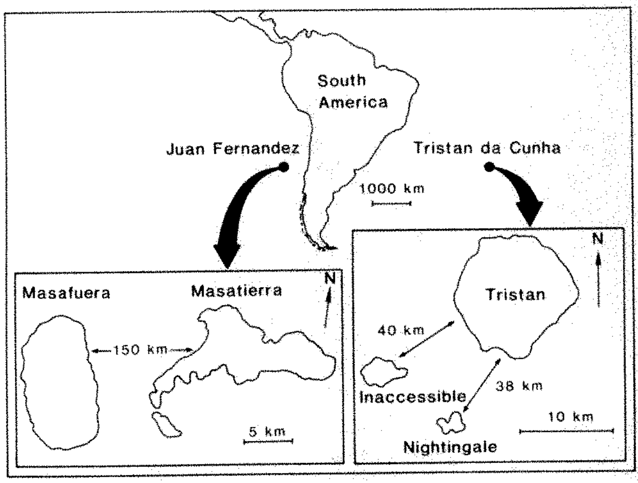

FIG. 1 Location of the Juan Fernandez and Tristan da Cunha archipelagos.

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representation of the prominent elements of the adjacent continents.

That convergent evolution accounts for the disjunct distribution of these two subspecies of *Peperomia* seems unlikely because both taxa are morphologically very similar[14] and sequester a similar array of compounds. This would mean that convergence would have occurred in both morphological and chemical features, which seems improbable.

Continental drift, plate tectonics, and ocean-floor spreading, also seem unlikely to be able to account for the distributional pattern of the subspecies of *Peperomia berteroana*. At the close of the Cretaceous (65 Myr) when the Piperaceae, as well as probably most flowering plant families, were in existence[15], about 800 km probably separated Africa and South America at their closest points, and neither the Juan Fernandez nor Tristan da Cunha Archipelago is older than 18 Myr. Therefore, the Tristan da Cunha Archipelago appeared when the South Atlantic Ocean was already opened to its full extent. Furthermore, the Juan Fernandez Islands have never been connected to continental Chile[6].

Long distance dispersal of the subspecies of *Peperomia berteroana* seems most concordant with all available information. One characteristic of the genus *Peperomia* that makes it suitable for this mode of dispersal is the production of sticky fruits[14,16], which can probably be dispersed among the feathers of migrating birds that visit both archipelagos. Such birds include *Fregetta grallaria* ssp. *melanoleuca* (the Tristan Storm Petrel), which has been recorded as breeding in both archipelagos[17], and *Pterodroma externa* (the Juan Fernandez Petrel), endemic to Masafuera, which probably only touches the Tristan Archipelago accidentally in its circumpolar wanderings. In addition, self-fertilization probably occurs in at least some species of the family Piperaceae[18,19], and would be advantageous for island colonization[20]. Furthermore, there is a marked similarity in the ecology on Tristan da Cunha and the Juan Fernandez archipelago; Masafuera as well as the islands in the Tristan da Cunha group have an alpine and subalpine flora[21].

The direction of long-distance dispersal seems to have been from Masafuera in the Juan Fernandez Islands to Inaccessible in Tristan da Cunha rather than the reverse. The genus *Peperomia* is represented by four species that are well-distributed on Masatierra and Masafuera; this is not so in Inaccessible where only one rare species exists, known only from the valley leading up from Salt Beach. Phylogenetic relationships based on morphological and flavonoid data[22] suggest that the populations on Tristan da Cunha are more derived than those of Juan Fernandez.

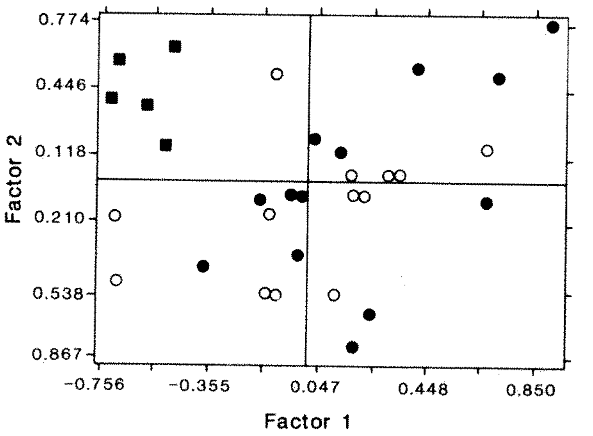

FIG. 2 Principal components analysis of *Peperomia* present in Juan Fernandez and Tristan da Cunha archipelagos. ●, *P. berteroana* ssp. *berteroana* (MT); ○, *P. berteroana* ssp. *berteroana* (MF); ■, *P. berteroana* ssp. *tristanensis*.

Even though several amphitropical Pacific examples in other genera exist (for example, *Rhamphogyne*, Compositae, with one species in the Mascarene Islands and another in New Guinea[23]) as well as disjunctions between Hawaii and the central-South Pacific (for example, *Charpentiera*, Amaranthaceae[24], and *Tetramolopium*, Compositae[25,26]), none of these disjunctions is between different oceans. The only other known flowering plant found in both archipelagos (as two different varieties) is *Empetrum rubrum* Vahl. (Empetraceae), which is also found in southern South America[27]. This example also may be due to long-distance dispersal by birds, but from a continental source[27]. □

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Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences

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LAKE Victoria, together with its satellite lakes, harbours roughly 200 endemic forms of cichlid fishes that are classified as 'haplochromines'[1,2] and yet the lake system is less than a million years old. This 'flock' has attracted attention because of the possibility that it evolved within the lake from one ancestral species[3] and that biologists are thus presented with a case of explosive evolution. Within the past decade, however, morphology has increasingly emphasized the view that the flock may be polyphyletic[4,5]. We sequenced up to 803 base pairs of mitochondrial DNA from 14 representative Victorian species and 23 additional African species.

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TABLE 1 Variation in the control region among 32 endemic fishes of the Lake Victoria flock

Species	No. of individuals	Bases at 15 variable positions
1. *Astatotilapia piceatus*	3	TGGTGCCATCACCGG
2. *Astatotilapia elegans*	1	....A....T..T..
3. *Astatotilapia nubilis*	1	...C......T.T..
4. *Ptyochromis sauvagei*	3	....C.....C.T..
5. *Ptyochromis xenognathus**	3	..A.......C.T.A
		A A
6. *Labrochromis ishmaeli**	3	............T..
		C T
7. *Platytaeniodus degeni*	3	.........T..T..
8. *Macropleurodus bicolor*	2	.A........C.T..
9. *Lipochromis obesus*	5	......TG....T..
10. *Neochromis nigricans*	4	............T..
11. *Prognathochromis longirostris*	1	............T..
12. *Prognathochromis dentex*	1	..A.........T..
13. *Prognathochromis paraguiarti*	1	........CT.TTA.
14. *Harpagochromis guiarti*	1	C...........T..

Variable nucleotide positions are indicated with numbers 1–15 in Fig. 1*a*; dots indicate identity of a nucleotide with the first species and letters below lines indicate that more than one nucleotide has been observed at this position in the polymorphic species (*). Specimens of species 1, 2 and 4–8 were collected in the Mwanza Gulf, Tanzania (southern Lake Victoria) and the others at Jinja, Uganda (northern Lake Victoria). For species 3, 13 and 14 only positions 84–440 were sequenced. Species 2 and 3 are not strictly endemic to Lake Victoria but also occur in its surrounding lakes, therefore supporting Greenwood's notion of a 'species superflock' of Victoria haplochromines. The species include eaters of algae (7, 10), insects (1, 2, 7), fish larvae (9), fish (11–14) and molluscs (4–6). All endemic haplochromine cichlids of Lake Victoria and the sand-dwelling cichlids of Lake Malawi (group A in Figs 1, 2) were once placed into four monotypic genera plus the genus *Haplochromis*. Greenwood[19,20] revised the taxonomy of the Lake Victoria haplochromines and assigned ~170 species into 20 endemic genera, reducing the number of species in *Haplochromis* to six. The use of the term 'haplochromines' here is not intended to imply any taxonomic rank assignments to this group of cichlids[6,14,19,20].

The flock seems to be monophyletic, and is more akin to that from Lake Malawi than to species from Lake Tanganyika; in addition, it contains less genetic variation than does the human species, and there is virtually no sharing of mitochondrial DNA types among species. These results confirm that the founding event was recent.

Mitochondrial DNA (mtDNA) sequences were obtained from 14 species from 9 endemic genera of the Lake Victoria flock. These included two of the four monotypic genera described by Greenwood[6] as well as other representatives of the five main ecological and morphological groups, namely eaters of insects, fish, fish larvae, molluscs and algae (Table 1). A 363-base-pair (bp) part of the cytochrome *b* gene and a 440-bp segment of mtDNA that bears part of the threonine transfer RNA gene, all of the proline tRNA gene and the most variable part of the control region were sequenced after enzymatic amplification[7] (Fig. 1). There is little variation in these mtDNA segments among the 32 specimens examined; only 15 sites out of the 803 surveyed show base-substitutional differences (Table 1). The average number of differences between pairs of the 16 mtDNA types found was less than that occurring within human populations in this part of the control region[8]. A comparable result was evident from electrophoretic comparisons of proteins encoded by nuclear genes[3,9]. Despite the low genetic variation, there was virtually no sharing of mtDNA types among species (Table 1), a result that is exceedingly unlikely to arise by chance. This finding seems to rule out the possibility that most of the morphological species in Lake Victoria are merely alternative morphs of a few biological species[10,11]. No length mutations were detected in this survey and no variation was found in either the cytochrome *b* or the tRNA genes. In addition, our findings contrast with studies of other fishes, which generally show more intraspecific variation in conservative regions of mtDNA than is observed in our intergeneric comparisons within Lake Victoria in the control region[12,13].

TABLE 2 Base differences in two segments of mtDNA from African cichlid fishes

	Species	Geographical origin	No. of base-substitutional differences B	C	D	E	F
A.	*Hemichromis*	W. Africa	55	57	55	55	58
B.	*Julidochromis*	L. Tanganyika	—	40	44	43	41
C.	*Astatoreochromis*	E. Africa	58	—	24	23	32
D.	*Buccochromis*	L. Malawi	63	38	—	7	18
E.	*Pseudotropheus*	L. Malawi	63	38	17	—	18
F.	'Haplochromines'	L. Victoria	63	45	37	36	—

Mean number of differences in a 363-bp segment of the cytochrome *b* gene (from Fig. 1*b*) appear above the diagonal and in a 440-bp segment bearing tRNA genes and the control region below the diagonal (from Fig. 1*a*). The cytochrome *b* region was sequenced for a total of 18 specimens, which included 3 from 3 species of the Neotropical genus *Cichlasoma*, 1 *Hemichromis bimaculatus* from West Africa, 1 *Julidochromis regani*, 1 *Astatoreochromis alluaudi* (from Lake Victoria; this species also occurs in rivers of East Africa), 1 *Buccochromis atritaeniatus* (Malawi haplochromine group A), 1 each of 3 species of the *Pseudotropheus tropheops* complex from Lake Malawi (often called 'mbuna', and here called haplochromine group B), and 8 specimens of 7 haplochromine species from Lake Victoria (1, 2 and 4–8 in Table 1). No variation was detected among the specimens from Lake Victoria or among haplochromines of group B from Lake Malawi. The control region was sequenced from 66 specimens, which included 1 *Julidochromis regani* and 1 *Lamprologus birchardi* from Lake Tanganyika (which differ by 53 base substitutions, Fig. 1*a*), 8 specimens of *Astatoreochromis alluaudi* from Lake Victoria (3 from southern localities in Tanzania and 5 from northern Uganda; no fixed differences were found between northern and southern populations), 9 specimens of 9 species of group A from Lake Malawi (variation not shown), 15 specimens from Malawi of 10 group B species (variation not shown), and the 32 Lake Victoria haplochromines from 14 species whose results appear in Table 1. The Malawi group A species were *Chilotilapia rhoadesi*, *Buccochromis atritaeniatus*, *Nimbochromis polystigma*, *Dimidiochromis compressiceps*, *Maravichromis labidodon*, *Champsochromis spilorhynchus*, *Protomelas annectens*, '*Haplochromis*' *diaboli* and *Sciaenochromis gracilis*[14]; the Malawi group B representatives consisted of eight specimens from seven undescribed species of the *Pseudotropheus tropheops* species complex[21] (P. Reinthal and A. M., unpublished data), 5 of *Pseudotropheus zebra* and 1 each of *Melanochromis auratus* and *Labeotropheus fuelleborni*. Consensus sequences appear in Fig. 1 for *Astatoreochromis* and Malawi groups A and B as well as for the Victoria haplochromines.

To place these Victorian cichlids in relation to other cichlids, we sequenced both mtDNA segments in 26 additional species (Fig. 1, Table 2). As shown in Table 2, the endemic Victoria haplochromine species, which differ from each other by an average of only 3 substitutions (Table 1), differ from those of Lake Malawi by 54 to 55 substitutions and by at least 77 substitutions from other species. Likewise, the two groups of haplochromines from Lake Malawi, which differ from each other by an average of 24 substitutions, differ by at least 54 substitutions from cichlids elsewhere. Genealogical analyses using *Hemichromis*, from West Africa, as an outgroup confirmed this strong indication of monophyly for the Victoria flock (Fig. 2). Some of the morphologically convergent forms (for example, *Macropleurodus* from Lake Victoria, and *Chilotilapia* from Lake Malawi) that served before to support the notion of polyphyly[5] are represented in this study; they confirm our monophyly conclusion. In addition, two species endemic to Lake Tanganyika (*Julidochromis* and *Lamprologus*) are more distantly related to the taxa from Lakes Malawi and Victoria than is *Astatoreochromis*. This nonendemic Victorian haplochromine is the sister group to the endemic cichlid faunas of both Lakes Victoria and Malawi.

The 24 members of the Lake Malawi flock surveyed fall into two monophyletic groups, sand-dwellers and rock-dwellers, here called haplochromine groups A and B, respectively (Fig. 2). As each of these two ecological groups seems to consist of about 200 species[14] and our sampling of the faunal diversity of Lake Malawi may not be adequate, it would be premature to propose the existence of an exact association between phylogenetic position and ecological group for all Malawian species.

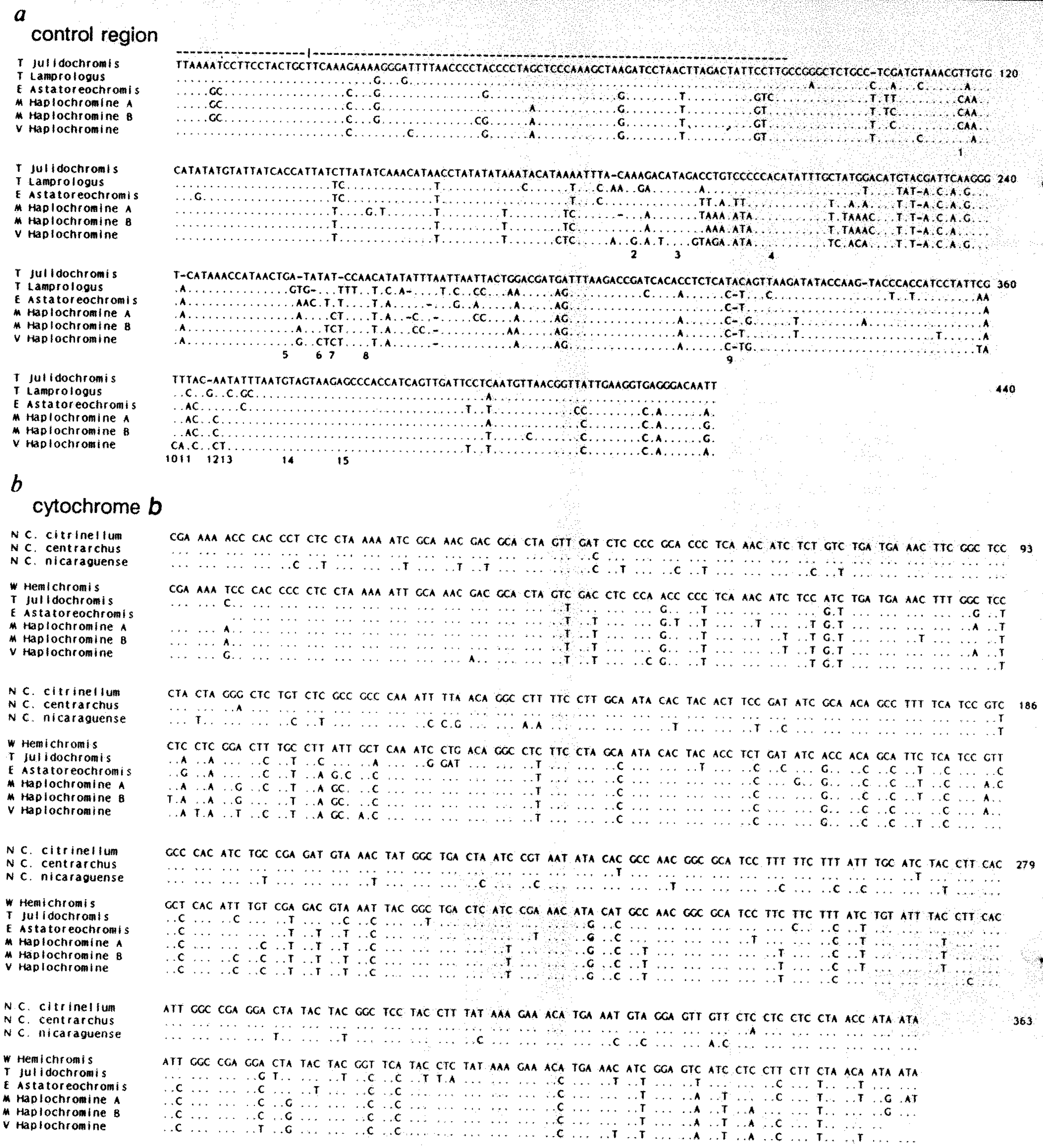
a
control region
T Julidochromis
T Lamprologus
E Astatoreochromis
M Haplochromine A
M Haplochromine B
V Haplochromine
TTAAAATCCTTCCTACTGCTTCAAAGAAAAGGGATTTTAACCCCTACCCCTAGCTCCCAAAGCTAAGATCCTAACTTAGACTATTCCTTGCCGGGCTCTGCC-TCGATGTAAACGTTGTG 120
CATATATGTATTATCACCATTATCTTATATCAAACATAACCTATATATAAATACATAAAATTTA-CAAAGACATAGACCTGTCCCCCACATATTTGCTATGGACATGTACGATTCAAGGG 240
T-CATAAACCATAACTGA-TATAT-CCAACATATATTTAATTAATTACTGGACGATGATTTAAGACCGATCACACCTCTCATACAGTTAAGATATACCAAG-TACCCACCATCCTATTCG 360
TTTAC-AATATTTAATGTAGTAAGAGCCCACCATCAGTTGATTCCTCAATGTTAACGGTTATTGAAGGTGAGGGACAATT 440
1
2 3 4
5 6 7 8 9
10 11 12 13 14 15
b
cytochrome b
N C. citrinellum
N C. centrarchus
N C. nicaraguense
W Hemichromis
CGA AAA ACC CAC CCT CTC CTA AAA ATC GCA AAC GAC GCA CTA GTT GAT CTC CCC GCA CCC TCA AAC ATC TCT GTC TGA TGA AAC TTC GGC TCC 93
CGA AAA TCC CAC CCC CTC CTA AAA ATT GCA AAC GAC GCA CTA GTC GAC CTC CCA ACC CCC TCA AAC ATC TCC ATC TGA TGA AAC TTT GGC TCC
CTA CTA GGG CTC TGT CTC GCC GCC CAA ATT TTA ACA GGC CTT TTC CTT GCA ATA CAC TAC ACT TCC GAT ATC GCA ACA GCC TTT TCA TCC GTC 186
CTC CTC GGA CTT TGC CTT ATT GCT CAA ATC CTG ACA GGC CTC TTC CTA GCA ATA CAC TAC ACC TCT GAT ATC ACC ACA GCA TTC TCA TCC GTT
GCC CAC ATC TGC CGA GAT GTA AAC TAT GGC TGA CTA ATC CGT AAT ATA CAC GCC AAC GGC GCA TCC TTT TTC TTT ATT TGC ATC TAC CTT CAC 279
GCT CAC ATT TGT CGA GAC GTA AAT TAC GGC TGA CTC ATC CGA AAC ATA CAT GCC AAC GGC GCA TCC TTC TTC TTT ATC TGT ATT TAC CTT CAC
ATT GGC CGA GGA CTA TAC TAC GGC TCC TAC CTT TAT AAA GAA ACA TGA AAT GTA GGA GTT GTT CTC CTC CTC CTA ACC ATA ATA 363
ATT GGC CGA GGA CTA TAC TAC GGT TCA TAC CTC TAT AAA GAA ACA TGA AAC ATC GGA GTC ATC CTC CTT CTT CTA ACA ATA ATA

FIG. 1 Mitochrondrial DNA sequences from *a*, part of the threonine tRNA gene (18 bp), the proline tRNA gene (71 bp) and a 350-bp segment of the control region of African cichlids and *b*, part of the cytochrome *b* gene for African and Central American cichlids. In *a*: dots, sequence identity with *Julidochromis*; dashes, deletions; 1–15, nucleotide positions that are variable among the endemic cichlids of Lake Victoria (see Table 1). In *a* and *b*, consensus sequences for all specimens sequenced are shown for the endemic Lake Victoria haplochromines (see Table 1), the haplochromine groups A and B from Lake Malawi, and *Astatoreochromis* (see Table 2). For position 342 in *a*, one species of the Malawi group A (*Buccochromis*) had an A inserted (as shown). The sequences in *a* correspond approximately to positions 15,937–16,497, and in *b*, to positions 14,759–15,121 in the human mtDNA sequence[22]. The gene arrangement in the cichlids is similar to that in sturgeon[23] but seems to differ from that in cod, in which a 74-bp insertion exists between the threonine and proline tRNAs[24]. In *b*: dots, sequence identity either with *Cichlasoma citrinellum* (one of three species of *Cichlasoma* sequenced from the Central American Neotropics) for the three *Cichlasoma* congeners or with *Hemichromis*, from West Africa, for the African taxa. Blanks, missing sequence; N, Neotropics; T, Lake Tanganyika; E, East Africa; M, Lake Malawi; V, Lake Victoria.

METHODS. DNA was extracted from tissues of frozen or ethanol-preserved specimens[7,8]. Amplifications were performed in 25-μl volumes of Tris buffer (67 mM, pH 8.8) containing 2 mM $MgCl_2$, 1 mM of each dNTP, 1 μM of each primer, 10–1,000 ng template DNA and *Taq* polymerase (1 unit; Cetus). The primers used for the cytochrome *b* gene were L14724 (5′-CGAAGCTTGATATGAAAAACCATCGTTG-3′, designed by S. Pääbo) and H15149 (ref. 7); for the control region they were L15926 (ref. 7) and H16498 (5′-CCTGAAGTAGGAACCAGATG-3′). L and H refer to the light and heavy strands, respectively, and the numbers refer to the position of the 3′ base of the primers in human mtDNA[22]. The temperature profile for 30 cycles of double-stranded and 35 cycles of single-stranded amplification was 45 s at 94 °C (denaturation), 1 min at 55 °C (annealing of primers), 2 min at 72 °C (polymerization). The rest of the protocol is reported in detail in ref. 7.

The patterns of base changes in the two regions sequenced behave as expected from surveys of other groups of vertebrates. Transitions outnumber transversions in both segments, and silent changes outnumber replacement changes in the cytochrome *b* gene. Moreover, the rate of change in the control region exceeds that in the cytochrome *b* gene. The results give no reason to suppose that the rates of mtDNA evolution are uneven or accelerated among East African cichlid fishes.

The likelihood of extreme recency for the Victorian flock is underscored by the finding that species within the Neotropical genus *Cichlasoma* differ by as much as 11% in their cytochrome *b* sequences[7] (Fig. 1*b*), whereas the Victoria flock differs by only 5% from the Malawi flock. Indeed, no diversity in the cytochrome *b* segment (legend to Table 2) was evident within the Victoria flock. An estimate of the divergence times among the flocks is based on the assumption that the mean rate of divergence in the cytochrome *b* gene is 2.5% per million years. Our molecular results date the origin of the extant Victorian flock at less than 200,000 years ago (Fig. 2). Lake Victoria's age has been estimated to be between 250,000 and 750,000 years[15], but younger ages are considered likely for the present-day fauna[3]. This date and the other estimated divergence times (Fig. 2) fit with the estimated geological ages of the three lakes (1-2 million years (Myr) for Lake Malawi and 2-4 Myr for Lake Tanganyika[16]). So it is likely that the Victoria flock arose within the lake.

Furthermore, this result weakens the hypothesis that similarly specialized species from different lakes are more closely related to each other than to morphological generalists from the same lake. These specializations probably evolved repeatedly and independently. The establishment of the monophyly of this species flock draws attention to the remarkable speed of the morphological diversification in Lake Victoria without an acceleration of molecular evolution.

Unfortunately, we are losing the opportunity to study the cichlid fauna of Lake Victoria, because much of it is going or has gone extinct as a result of the introduction of a non-endemic predatory fish.[17,18] ☐

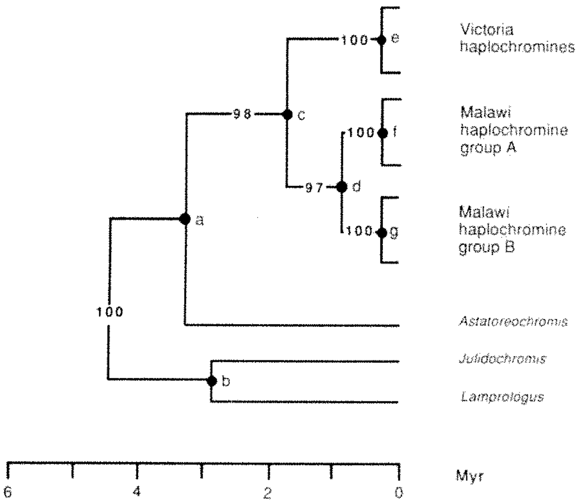

FIG. 2 Evolutionary tree for 36 African cichlid species based on a parsimony analysis[25] of mtDNA sequences. The tree is a composite based on two approaches. Only amino-acid replacement changes in cytochrome *b* were used to confirm the two deepest nodes, a and b, as well as to place the root on the ab branch, using *Hemichromis* as an outgroup. The four equally short trees obtained (each with 14 steps, consistency index CI = 0.75) did not sort out the branching order inside the group stemming from node a. A second approach used control region data with two cichlids of Lake Tanganyika (*Julidochromis* and *Lamprologus*) as the outgroup and established the branching pattern shown, with high boot-strap values (97-100)[26] confirming each of the internal branches (ab, ac, cd, ce, df and dg). The data did not allow the resolution of the relationships within any of the three assemblages stemming from nodes e, f and g (accordingly, parsimony analysis yielded nine equivalent solutions, each requiring 178 mutations; CI = 0.826). Other studies suggest that the cytochrome *b* gene diverges at a rate of at least 2.5% per million years in mammals[29]. On the basis of this rate and the per cent differences obtainable from Table 2, *Astatoreochromis* shared a common ancestor with the endemic Lake Victoria cichlids ~3.5 Myr ago, in exact agreement with the date inferred from studies of proteins encoded by nuclear genes[3]. The two main groups of cichlid fishes from Lake Malawi seem from this approach to have had a common ancestor ~700,000 years ago, the geological age of this lake being 1-2 Myr[16]. The low genetic distances observed among the proteins of the Malawi haplochromines B are consistent with this inference[27,28].

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Transparency and the uniqueness constraint in human and computer stereo vision

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THE sensation of depth that is obtained with human binocular vision results from the differences in the projection of the world onto the two retinae. The process entails solving the problem of stereo correspondence, which involves choosing the correct matches between left and right image features. Many computational models of stereo vision assume a uniqueness constraint on stereo matching—that is, each feature identified in one image should eventually be matched with only one feature in the other image[1,2]. This constraint would seem to be justified, as allowing non-unique matches would be tantamount to supposing that the scene entities to which matches relate are in two places at once[1]. The value of the uniqueness constraint for eliminating false matches has been demonstrated in a variety of stereo algorithms. Yet on the basis of psychophysical results Weinshall[3] concluded that it was not used by humans in dealing with certain types of ambiguous random-dot stereograms. We have now tested how Weinshall's stereograms are dealt with by PMF[4], a stereo algorithm which uses a

unique-matches selection procedure in conjunction with a purely local similar-disparity support scheme. We found that PMF produces results that are closely analogous to the psychophysical results. This suggests that Weishall's experiments should not be interpreted as evidence that the human stereo mechanism establishes non-unique matches.

With the PMF algorithm, support for each potential match is sought within a local neighbourhood from other potential matches that share similar disparities. Similarity is defined in terms of the disparity between potential matches not exceeding a moderate disparity gradient limit[5]. (Psychophysical measurements suggest that the human visual system is limited by a disparity gradient of about unity for allowable binocular fusion[6]. We exploited the disparity gradient concept to establish stereo correspondences. We view[5] the magnitude of the disparity gradient limit in PMF's local support scheme in terms of a trade-off between disambiguation power and the ability to deal with as wide a class of scene structures as possible. Gradient limits less than unity provide greater disambiguating power, and are often therefore preferable for finding matches, but at the

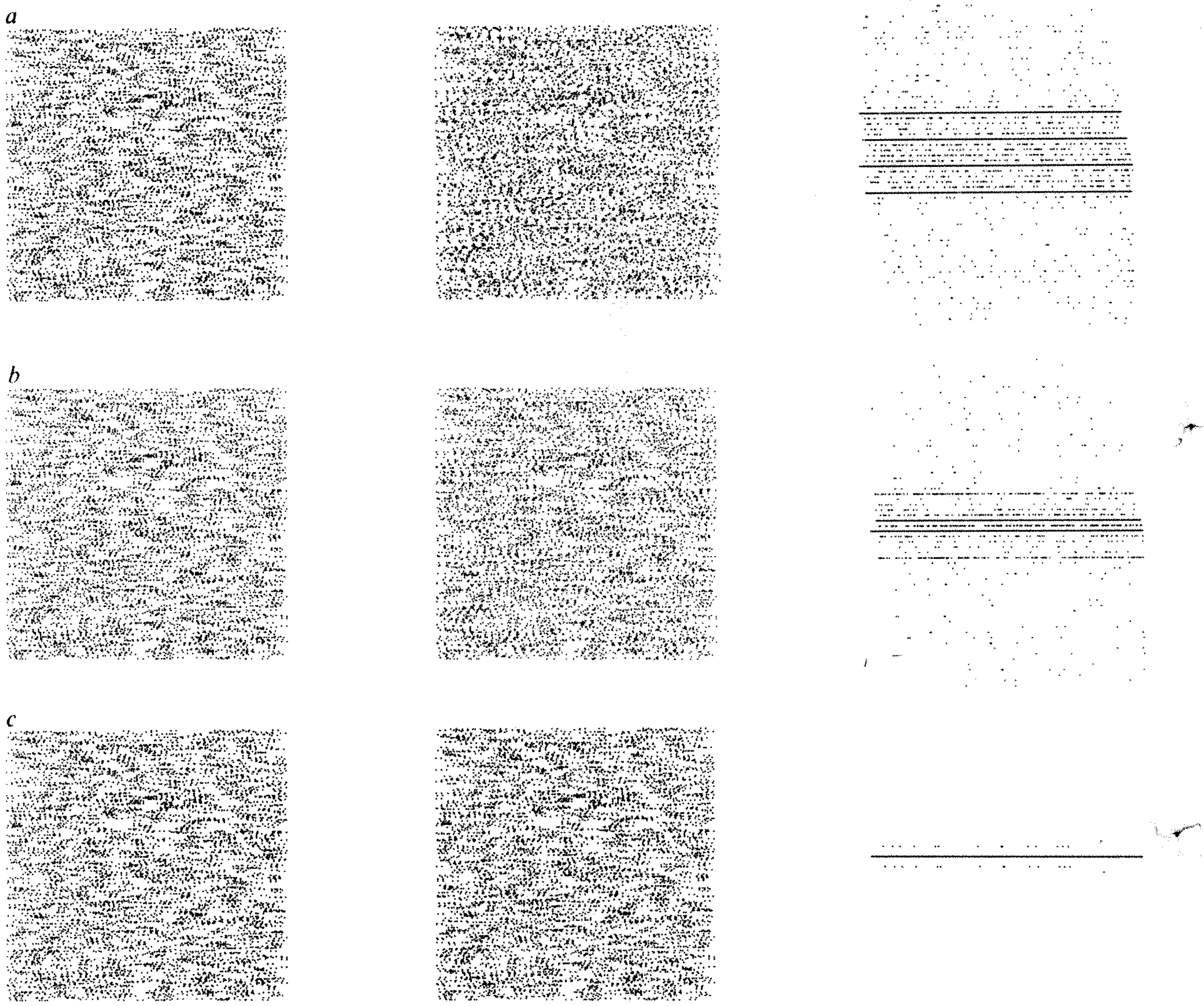

FIG. 1 Three random-dot stereograms and the results of the application of PMF to each of them. Each stereogram is constructed as follows. A 5% random dot pattern (256 units square) is replicated in the left image with a spacing between copies of G_l pixels and in the right image with spacing G_r pixels. The right-hand column shows the respective profiles through disparity space of the unique matches identified by PMF: for purposes of illustration, all matches from all over the associated stereo pair are compressed into a single cross-section, with disparity planes varying from convergent to divergent from bottom to top of the figure. For the experiments reported here, PMF's support neighbourhood was defined as a circular patch of radius 10 pixels in the left image, and the disparity range for the pool of potential matches was +/−20 pixels. In *a*, $G_l=5$ and $G_r=10$, unique matches were obtained by PMF (when run with a limiting disparity gradient of 0.5) for 5,814 of the possible 6,246 image primitives. These were distributed densely within four disparity planes so that they appear as almost solid lines in the profile figure. Psychophysical data[3] for stereograms similar to *a* are similar in that some human observers report stable fusion of up to four transparent depth planes. The inner pair of dense planes (at disparities of 0 and 5 pixels)—called the 'correct solution' in the text because they have the disparities of the two copies of the random dot texture—comprise only slightly more selected matches (1,330 and 1,226 uniquely determined matched points, respectively) than the outer pair of dense 'ghost' planes at 10 and −5 unit disparities (1,113 unique matches in each plane). This distribution reflects the marginally increased chance of matches between the correct planes exchanging support than in the ghost cases. If the disparity gradient limit used in PMF is increased to 1.0 (the psychophysical limit of fusion) qualitatively similar results are observed but the number of unique matches in the correct planes increases (1,840 and 1,716) and in the ghosts planes decreases (390 and 447). *b* ($G_l=5$ and $G_r=7$) shows that as the disparity difference between disparate copies is decreased the correct solution becomes dominant: in this case, PMF can match uniquely 5,951 matches of which most (2,351 and 2,523) exist in the inner pair of planes (at disparities of 0 and 2 pixels, respectively). *c*, Illustration of the way in which PMF selects a single surface (with 6,159 unique matches in it and a few incorrect fusions at disparities of 1 or 2 pixels from it) for the condition where $G_l=G_r=5$. This result is also in close accord with psychophysical data[3].

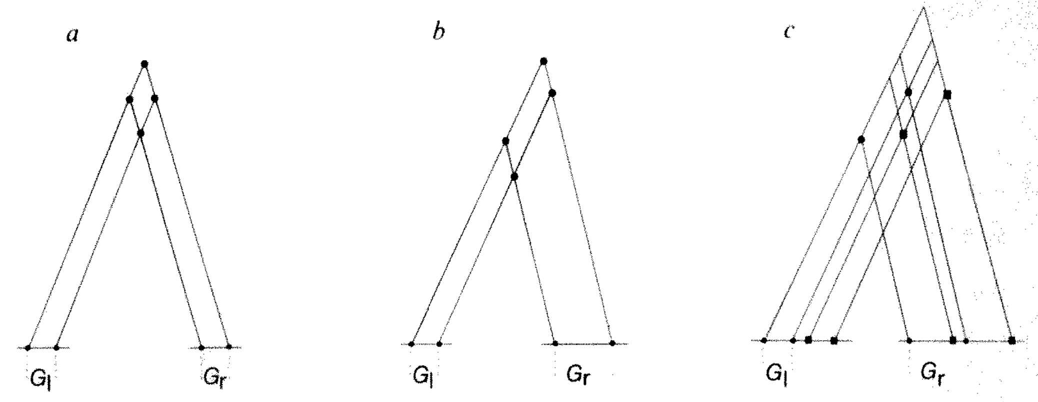

FIG. 2 Variants of the double nail illusion. *a*, Pairs of identical objects presented to humans are generally seen as lying side by side when they originate from thin objects (such as nails, needles or pins) arranged one immediately behind the other. The 'correct' solution would require a change in the order of projection of points along matching epipolar lines in the two images[7]. *b*, Asymmetric version of the same illusion. Again the order-preserving solution is preferred. *c*, Double double nail illusion. Two sets of pairs of points in the left and right images with separations G_l and G_r between copies and with $G_r = 2G_l$. If the separation in the left image between the circular and square primitives is less than G_r then a change of order in the right image results.

price of less good performance on three-dimensional surfaces varying rapidly in depth.) After calculation of support scores, matches are selected according to a winner-take-all scheme in which maximally supported matches are chosen such that only one match is permitted for each left and right image feature. This final selection stage imposes the uniqueness constraint. One important feature of the algorithm is that no cost is imposed from neighbouring matches that violate the disparity gradient limit (compare with Prazdny's similar algorithm[7]). Hence PMF can deal (to an extent dependent on particular image characteristics, see below) with partially transparent surfaces that are only locally spatially cohesive[4,7].

Figure 1 presents examples of the kind of random dot stereograms used by Weinshall and the results of applying PMF to them. The stimuli were created by first generating a random dot pattern and then constructing each stereo image by adding another copy of it, with horizontal spacing between copies in the left and right images being termed G_l and G_r respectively. Both PMF and (some) human observers interpret stereograms like Fig. 1*a*, in which $G_r = 2G_l$, as consisting of four transparent planes of dots. When $G_r = G_l$, as in Fig. 1*c*, both PMF and humans report a single plane. Computational experiments with stimuli of this kind have revealed that the number of planes appearing in the output from PMF is dependent on a complex interaction between: (1) the disparity difference between copies in the left and right images (increasing the difference strengthens the ghost planes: for example, an intermediate condition in which $G_r = 1.4G_l$ produces two planes, Fig. 1*b*); (2) the size of the limiting disparity gradient used in the computation of local support (increasing the limit weakens the ghost planes); and (3) density of the stereogram (increasing the density strengthens the ghost planes).

Weinshall[3] argued that the imposition of the uniqueness constraint for Fig. 1*a* should result in matches lying in just two disparity planes, one for each copy of the random dot pattern from which the stereo halves were made (these two planes will hereafter be called the 'correct' planes). At first sight this seems a reasonable claim because arriving at four planes suggests that each copy is being matched more than once. Yet the prediction rests on the assumption that each and every match reflecting the disparate positions of the two copies should obtain more support than competing 'incorrect' matches lying in other so-called 'ghost' planes. This assumption is false for an algorithm such as PMF, which incorporates only local support. The complex patterns of interference that arise between random dots in each of the potential transparent surfaces, correct and incorrect, are such that at least some incorrect matches will obtain more local support (and hence will be uniquely selected) than their correct counterparts.

Why do fewer planes emerge as the disparity difference decreases between copies in each image? The decrease leads to an increase in the probability of support existing between the correct disparity planes relative to the probability of equivalent support existing between other combinations of disparity planes. The decrease also leads to a decrease in the interference between the local support structures for dot patterns in each plane. Together these factors cause the correct solutions to become increasingly dominant with decreasing disparity difference, with unique solutions tending to be consistent with the two correct surfaces for certain values of separation (see, for example, Fig. 1*b*). The limiting case is when the disparity difference is decreased to zero, which produces a single coherent surface (as in Fig. 1*c*).

Weinshall's stereograms are designed to be random-dot extensions of the double nail illusion[8]. The latter arises from just two elements in each stereo image (Fig. 2). The reported psychophysical results, and those obtained from the application of PMF, to the random dot versions, however, reveals important differences between the two cases. At the level of dot micropatterns, it is clear that when considered in isolation, each repeated dot couplet along matching rasters in the left and right random dot images forms an individual version of a stimulus pattern for generating the double nail illusion. But at the macro level of dense random dot patterns portraying transparent surfaces, complex interactions and ambiguities occur that cannot be accounted for by treating these stimuli as enlarged versions of the double nail case. For example, consider the situation illustrated in Fig. 2*c*, which shows how the ordering of dots along a pair of matching rasters fails to be preserved because of an interaction between just a pair of double dot couplets.

Contrary to Weinshall, we conclude that just because the kind of stereogram shown in Fig. 1*a* can produce the perception of four distinct depth planes, the human visual system does not necessarily *ipso facto* fail to impose the uniqueness constraint. Indeed, the phenomenon of four depth planes supports exactly the opposite conclusion, at any rate for dense textures, insofar as a stereo algorithm that imposes unique matches also produces four depth planes for equivalent stimuli.

It would be possible in principle to devise a stereo algorithm that imposed unique matches and also produced just two depth planes for Fig. 1*a* and *b*. Such an algorithm would need to incorporate a benefit for unique matches lying in the minimum number of globally cohesive planes. The human visual system does not produce the minimum number of planes consistent with unique matches and this suggests that it may use solely local neighbourhood support interactions of the kind intrinsic to the design of PMF. ☐

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Reformation of long axon pathways in adult rat central nervous system by human forebrain neuroblasts

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THE failure of lesioned axons to regenerate over long distances in the mammalian central nervous system (CNS) is not due to an inability of central neurons to regenerate, but rather to the non-permissive nature of the CNS tissue environment[1–4]. Regenerating CNS axons, which grow well within a peripheral nerve, for example, fail to penetrate mature CNS tissue by more than about 1 mm[1,5,6]. Recent evidence indicates that this may be due to inhibitory membrane proteins associated with CNS oligodendrocytes and myelin[2–4,7,8]. We report here that human telencephalic neuroblasts implanted into the excitotoxically lesioned striatum of adult rats can escape or neutralize this inhibitory influence of the adult CNS environment and extend axons along major myelinated fibre tracts for distances of up to ~20 mm. The axons were seen to elongate along the paths of the striato-nigral and cortico-spinal tracts to reach the substantia nigra, the pontine nuclei and the cervical spinal cord, which are the normal targets for the striatal and cortical projection neurons likely to be present in these implants.

Rats subjected to a unilateral ibotenic acid lesion of the caudate-putamen were injected into the same area after 7–10 days or one year later with a cell suspension prepared from the telencephalic ganglionic eminences ($n = 16$) or from the rhombencephalic lip ($n = 4$) dissected from the brains of 8–10 week-old aborted fetuses (Fig. 1). The rats were immunosuppressed to prevent rejection of the xenogeneic tissue[9]. Brains were processed for immunohistochemical analysis 12–25 weeks after implantation (Fig. 1). The human neurons and their associated axonal outgrowth were visualized using a species-specific antibody raised against a constituent of human neurofilaments (HNF) that has a molecular weight of 70,000 (refs 10, 11). Control sections from intact or ibotenic acid-lesioned rat brains were completely unstained by this antibody. This was also the case for sections from transplanted brains, identical to the ones used here but carrying implants of fetal rat or mouse ganglionic eminence tissue.

Fourteen of the 20 grafted rats (12 telencephalic and 2 rhombencephalic grafts) contained viable HNF-positive implants. Nissl-stain revealed patches of mature-looking graft neurons interspersed with areas of small, immature-looking cells. The HNF-immunoreactivity was primarily confined to densely stained fibres within the well developed areas of the implants. Adjacent sections stained with antibody against the dopamine D1 receptor-related phosphoprotein DARPP-32, used as a marker for striatal projection neurons[12], revealed DARPP-32-positive and DARPP-32-negative patches within the neuron-rich areas of the telencephalic implants. This indicates that, like grafts of rat ganglionic eminence tissue[13,14], the present human telencephalic implants were composed of a mixture of striatal and non-striatal (probably, at least in part, cortical) tissue.

In seven of the brains with surviving telencephalic implants, extensive outgrowth of HNF-positive fibres occurred within the host brain (Fig. 1). The fibres could be traced in large numbers, both along the internal capsule and the cerebral peduncle caudally towards the brain stem, and along the corpus callosum and the deep layers of the cerebral cortex into large areas of the overlying cortex. The predominant fibre outgrowth was along the myelinated fibre tracts and at higher magnification (Fig. 2*a* and *b*), the HNF-positive fibres could be seen to run both within and on the surface of the individual host myelinated fibre fascicles. This long-distance fibre outgrowth also occurred in the two animals in which the striatal lesion had been made one year before the fetal cell implantation. By contrast, very little fibre outgrowth was observed from the rhombencephalic tissue implants, despite the fact that they were themselves rich in HNF-positive fibres.

The caudally directed fibres extended in slender fascicles across the caudal and medial aspects of the implant–host border, through the adjacent host striatal neuropil to join the caudally running fibre bundles of the host internal capsule (Fig. 2*d*). The HNF-positive fibres coursed caudally into and through the globus pallidus, where some of them branched or turned into this nucleus (Fig. 1*a*, and levels II and III in Fig. 1*b*). A few fibres deviated into the thalamus. Interesting branching patterns were seen, such as the one depicted in Fig. 2*c*, where the principal axon gave off a collateral branch that passed through the bundle of white matter to ramify among the cell bodies within the globus pallidus. The fibres continued as a well defined bundle in the medial part of the internal capsule, in a position similar to that of the striato-nigral and cortico-spinal pathways; along the way, axons also branched off into the subthalmic nucleus (Fig. 2*e*). In the region of the substantia nigra, some axons turned dorso-laterally into the overlying pars reticulata; others continued in the medial part of the cerebral peduncle (levels V–VI in Fig. 1*b*; and Fig. 2*f*) and could be traced in high numbers into the pontine nuclei at a distance of 8–10 mm from the implant. Some fibres were also identified in deep parts of the tectum. In the cerebral peduncle, the HNF-positive fibre bundles occupied a position similar to that of cortico-fugal axons labelled anterogradely from the fronto-parietal cortex in intact animals[15,16]. In two of the animals, where the lower brain stem and upper spinal cord were included in the analysis, HNF positive fibres were observed in the medulla oblongata at the level of the pyramidal crossing, and in the white and grey matter of the upper cervical spinal cord at a distance ~20 mm from the implant.

The retrograde fluorescent tracers Fluoro-Gold and rhodamine-labelled latex beads were injected into the globus pallidus and substantia nigra, respectively, in two rats with telencephalic implants (Fig. 1). Clusters of Fluoro-gold-positive cell bodies and scattered cells labelled with rhodamine-latex beads were identified within the mature-looking regions of the implants, thus confirming that neurons in the implants projected to these sites.

The fibres projecting into the cortex extended in large numbers along the corpus callosum, the external capsule and the forceps minor, and within the deep layers of the cortex bilaterally. Labelled fibres were distributed in the frontal, parietal and occipital cortices on both sides. Ventrally, HNF-positive fibres could be traced into the amygdaloid-piriform area at about 8–10 mm away from the implants.

In line with the idea that the formation of long fibre tracts in the CNS reflects an interaction and balance between growth-promoting and growth-inhibiting factors along the growth trajectory, it has been proposed[2,4,7] that the limited regrowth of axotomized neurons in the adult brain or spinal cord is, at least in part, due to an active inhibition of axonal elongation exerted by the oligodendrocytes and myelin components of mature white matter, and that blockade of this inhibition can markedly improve axonal regeneration[4]. At least certain types of immature CNS neurons may be relatively insensitive to these growth-inhibiting factors during a limited period of development. This period may coincide with the time of active axonal elongation and pathway formation when growing axons are known to have special growth substrate requirements[17,18]. The human neuroblasts, which have a very protracted development, are likely to remain in their phase of active axonal elongation for a long period after implantation, thus making it possible for them to extend axons over long distances in the lesioned host brain. Mouse telencephalic neuroblasts, with a shorter developmental time span, show more restricted axon outgrowth (as visualized with a mouse-specific antibody) when grafted into the rat striatum under identical (immunosuppressed) conditions (K.W. *et al.*, submitted). Indeed, with implants of fetal rodent neurons, extensive axonal projections in lesioned adult brains have previously been observed only when the implants are placed close (generally <1 mm) to the target[19-26]. This difference in growth capacity may also reflect the large difference in size between rodent and human neurons: the length of, for example, the striato-nigral projection in the human brain (~30 mm) is ~10 times longer than that of the mouse. The considerably larger dimensions of the pathways normally formed by the human telencephalic neuroblasts may thus be expressed as a greater growth capacity in the implant situation as well.

Axonal growth from the telencephalic grafts showed a remarkable selectivity, and it was specific in the sense that it did not occur from identically positioned rhombencephalic neuroblasts. The descending HNF-positive axons formed a well defined bundle at a position closely matching that of the striato-nigral and cortico-ponto-spinal pathways in the intact brain and they branched off into nuclei normally densely innervated by these pathways.

These results indicate that the basic requirements for the reformation of long axonal pathways may be present in the lesioned adult mammalian CNS, provided that the regrowing

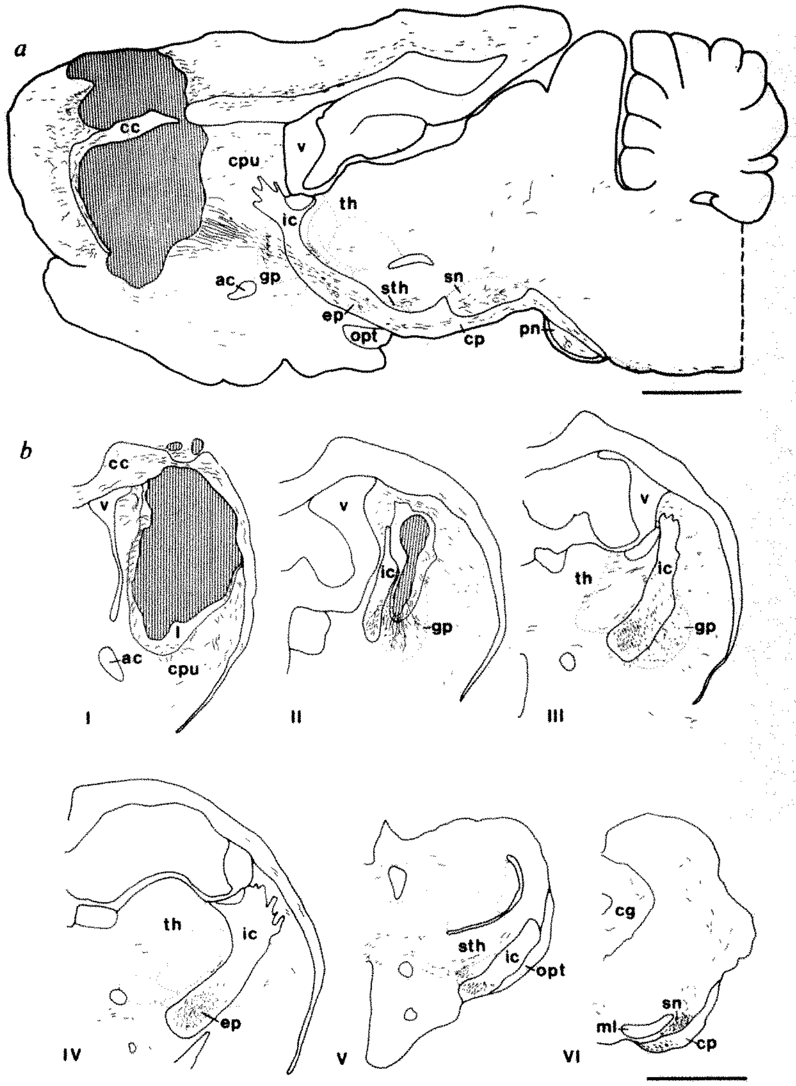

FIG. 1 Semischematic drawings of HNF-stained sections from adult rat brains with intrastriatal implants of human fetal ganglionic eminence tissue, showing the extent of the HNF-positive efferent projections. *a*, Drawing of 3–4 superimposed sagittal sections from a rat with a 23-week-old graft (hatched area). *b*, Coronal sections from 6 different rostral-to-caudal levels, 21 weeks after implantation. ac, Anterior commissure; cpu, intact caudate-putamen; cc, corpus callosum; cp, cerebral peduncle; ep, entopeduncular nucleus; gp, globus pallidus; ic, internal capsule; l, lesioned host caudate-putamen; opt, optic tract; pn, pontine nuclei; sth, subthalamic nucleus, sn, substantia nigra; th, thalamus; v, lateral ventricle. Scale bars, 2 mm.

METHODS. A total of 20 adult female Sprague-Dawley rats (200 g body weight) were anaesthetized with Equithesin ($0.3\ \mathrm{ml}\ 100\ \mathrm{g}^{-1}$), placed in a Kopf stereotaxic frame, and unilaterally injected into the head of the caudate-putamen with 14 μg ibotenic acid (Sigma; $10\ \mu\mathrm{g}\ \mu\mathrm{l}^{-1}$) as previously described[25]. One to two weeks later the rats received injections into the lesioned area of cell suspensions prepared either from the ganglionic eminences ($n = 14$) or the rhombencephalic lip ($n = 4$), dissected from the brains of aborted human fetuses of 8–10 weeks postmenstrual age. Two additional animals received implants of ganglionic eminence tissue at 1 yr after the ibotenic acid lesion. With the approval of the Research Ethical Committee at the University of Lund, tissue was retrieved from the fragmented material of 8 fetuses from suction abortions. Cell suspensions were prepared as before[27]. In brief, the dissected pieces were collected in 0.6% glucose-saline, incubated with 0.1% trypsin (20 min, 37 °C), rinsed and triturated into a milky cell suspension through the tip of a fire-polished Pasteur pipette to a total volume of 50–100 μl for each dissected region. Between 6 and 9 μl suspension, containing $\sim 2\text{–}4 \times 10^5$ viable cells, were injected into each of the rats, and divided over 2 or 3 injection sites. From the day of implantation, rats were immunosuppressed by daily injections of cyclosporin A (Sandoz; $10\ \mathrm{mg\ kg}^{-1}$, i.p.)[9]. Despite this immunosuppressive treatment, about one third (6 out of 20; 4 with telencephalic, and 2 with rhombencephalic grafts) of the grafts were rejected. At 12–25 weeks after transplantation, the rats were deeply anaesthetized with chloral hydrate ($350\ \mathrm{mg\ kg}^{-1}$), and perfused through the ascending aorta with 50 ml saline at room temperature, followed by 300 ml ice-cold phosphate-buffered 4% paraformaldehyde. After 2 h of postfixation and overnight incubation in 20% sucrose, brains were cut on a freezing microtome (30 μm) in the sagittal or coronal planes. Sections were incubated for 1 h with 10% normal horse serum in 0.02 M potassium phosphate-buffered saline, followed by a 48-h incubation with a mouse primary antiserum specifically recognizing the 70K polypeptide (molecular weight, 70,000) constituent of human neurofilaments (Serotec, UK) at a dilution of 1:200 in potassium phosphate-buffered saline with 2% normal horse serum, and 0.3% Triton-X100. To test the specificity of the human neurofilament antiserum, control sections from intact or lesion-only rats, and from rats with fetal rat or mouse implants, were processed according to the same protocol. An avidin-biotin-peroxidase complex system (Vector) was used to detect the primary antiserum, with 3,3′-diaminobenzidine as chromogen, and osmium-intensification of the reaction product. Alternate sections were incubated with a primary antiserum raised (in mouse) against DARPP-32 at a dilution of 1:20,000, and reacted according to the same protocol, except that no Triton-X100 was used. Two of the rats with telencephalic grafts received injections from micropipettes of the retrograde tracers Fluoro-Gold (Fluorochrome Inc.) and rhodamine-labelled latex beads (Lumafluor Inc.) into the host brain globus pallidus and substantia nigra, respectively, at 1–2 week before death[25].

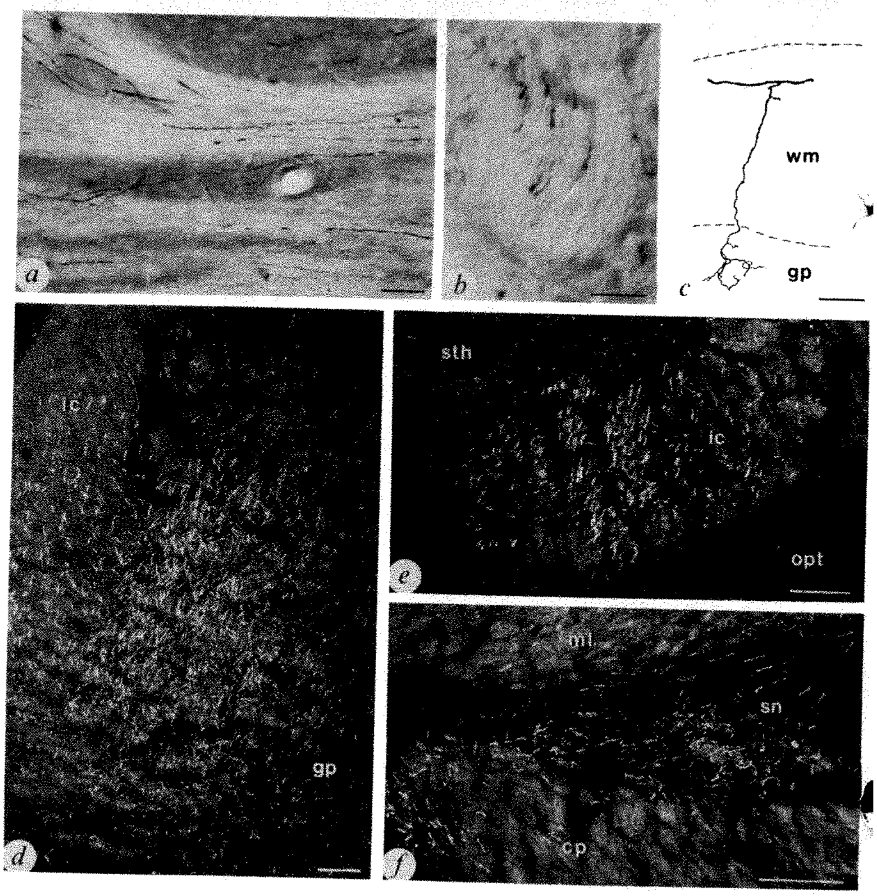

FIG. 2 Details of the HNF-immunoreactive efferent projections extending into the host brain from implants of ganglionic eminence tissue. *a* and *b*, High-power brightfield micrographs from sagittal and coronal sections, respectively, showing the position of the efferent projecting HNF-immunoreactive fibres along and inside the white matter bundles of the host internal capsule. *c*, High-magnification camera lucida drawing from a sagittal section illustrating how a thin axonal branch extended from a thicker main axon running within a white matter (wm) bundle, into the adjacent globus pallidus (gp), where it ramified among the host cell bodies. This branching pattern was often found in this region in the animals with telencephalic implants, and also in the rats with 1-year-old lesions at the time of implantation, as illustrated here. *d–f*, Darkfield micrographs from coronal sections corresponding to levels III, V, and VI, respectively, of the drawings in Fig. 1*b*. *d*, Section from the level of the rostral globus pallidus (gp) showing large numbers of HNF-positive efferents from the graft extending along and inside the host internal capsule (ic) bundles. At this level, some fibres were seen to branch off into the gp. *e*, The fibres continued caudally in the medial part of the internal capsule (ic), with some fibres running off into the region of the subthalamic nucleus (sth). *f*, Further caudally, some HNF efferents extended into the substantia nigra (sn), and others continued in the medial part of the cerebral peduncle (cp) to reach even more caudal host brain regions. Scale bars: *a*, 50 μm; *b* and *c*, 25 μm; *d–f*, 200 μm.

axonal elements can escape or neutralize the inhibitory features of the growth trajectory. Extensive axonal outgrowth from implanted fetal neurons in the adult brain depends on the presence of a preceding lesion[23,26]. However, the observation that the long fibre outgrowth was also present when the telencephalic cells were implanted one year after the striatal lesion indicates that the necessary guidance and growth-promoting mechanisms persist in the chronically lesioned CNS as well. The extensive pathway-forming capacity of the slowly developing human neuroblasts should provide new possibilities for the analysis of the cellular interactions underlying successful regeneration in the CNS. ☐

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The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor α gene to a novel transcribed locus

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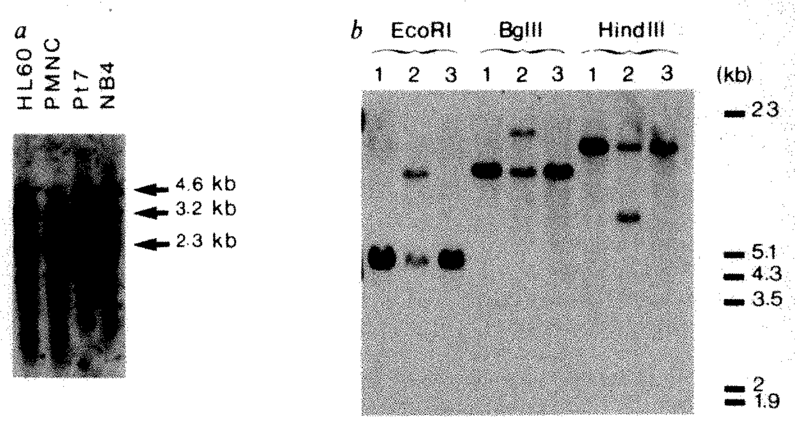
RETINOIC acid is a vitamin A derivative with striking effects on development and cell differentiation[1–3]. Several nuclear retinoic acid receptors (RARs), acting as ligand-inducible transcription factors, have been characterized[4–8] and indirect evidence suggests that they have distinct roles[9–11]. One of the most intriguing properties of retinoic acid is its ability to induce *in vivo* differentiation of acute promyelocytic leukaemia (APL) cells into mature granulocytes, leading to morphological complete remissions[12–13]. Because the *RARα* gene maps to chromosome 17q21 (ref. 14), close to the t(15;17) (q21–q11–22) translocation specifically associated with APL[15], we analysed *RARα* gene structure and

FIG. 1 *a*, Retinoic acid receptor α gene expression in patient 7 and in the NB4 APL cell line. Northern blot analysis was performed with an *RARα* cDNA probe[5] using 5 μg of poly(A)$^+$ RNAs extracted from the human myeloid leukaemic cell line HL60, human peripheral blood polymorphonuclear cells (PMNC), fresh leukaemic cells from patient 7 (Pt7) and the NB4 cell line derived from blast cells of patient 7. *b*, Rearrangement of the *RARα* gene in NB4 cells. Genomic DNAs from NB4 (lane 2) as well as from two controls, PMNC (lane 1) and HL60 (lane 3), were digested with the indicated restriction enzymes. The Southern blot was hybridized to an *RARα* cDNA probe specific for the second exon (V. Giguère, personal communication) which encodes the B and C1 domains[24]. The 147-bp fragment used as a probe was synthesized using PCR[25] and two 16-mer oligonucleotides (positions 406 and 553 in ref. 5) as primers. The DNA markers are aligned.



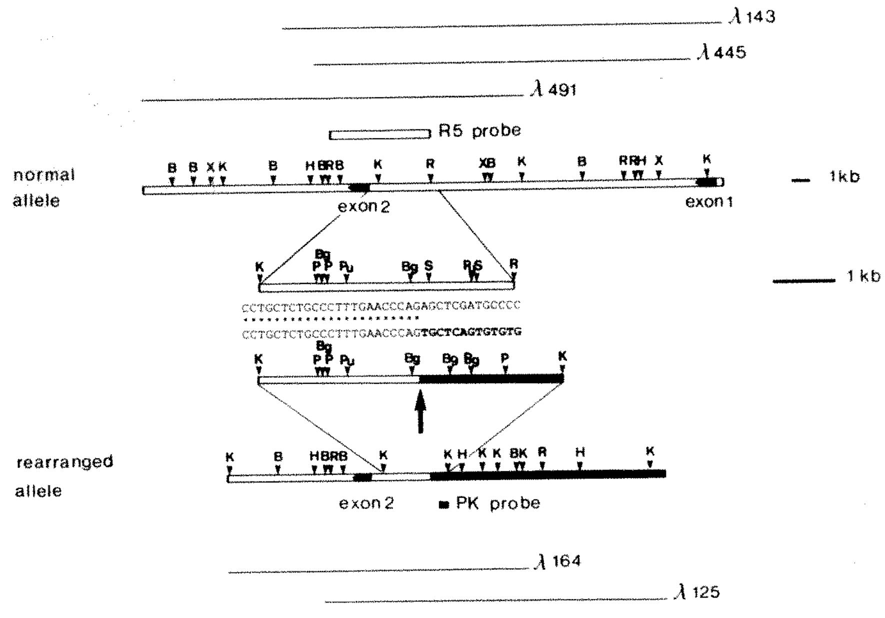
expression in APL cells. We report here that, in one APL-derived cell line, the *RARα* gene has been translocated to a locus, *myl*, on chromosome 15, resulting in the synthesis of a *myl/RARα* fusion messenger RNA. Using two probes located on either side of the cloned breakpoint, we have found genomic rearrangements of one or other locus in six patients out of eight, demonstrating that the *RARα* and/or *myl* genes are frequently rearranged in APL and the breakpoints are clustered. These findings strongly implicate retinoic acid receptor α in leukaemogenesis.

Preliminary studies suggested that the *RARα* gene was rearranged and its expression altered in APL patients (C.C. and P. Ballerini, personal communication). To establish this, bone marrow cells were collected from a t(15;17)-positive APL patient (Pt7) and a stable promyelocytic cell line (NB4) was derived from a primary culture of the blast cells (M.L. *et al.*, submitted). Expression studies in the fresh leukaemic cells and the cell line revealed an abnormal 4.6-kilobase (kb) *RARα* mRNA in both (Fig. 1*a*). Southern blot analysis revealed an additional *Eco*RI, *Bgl*II and *Hin*dIII restriction fragment in the DNA of Pt7 and NB4, compared with nonleukaemic cells, indicating that the *RARα* gene was identically rearranged in both samples (not shown). Using several subregions of the *RARα* complementary DNA as probes, we demonstrated that a short 147-base pair (bp) DNA fragment, corresponding to exon 2 (V. Giguère, personal communication), detected the DNA rearrangement (Fig. 1*b*) whereas the other probes did not.

A genomic library was generated from NB4 DNA and hybridized to the radiolabelled probe for *RARα* exon 2. Three clones (λ143, λ445, λ491) correspond to 28 kb of germline DNA, as shown by the sizes of hybridizing restriction fragment (Fig. 2). On the genomic map, the presence of the first and second α exons defines the direction of transcription. Two other overlapping clones (λ125, λ164) cover 21 kb of DNA, of which only 10 kb share a common restriction map with the germline *RARα* gene (Fig. 2), suggesting that these two λ phages originate from the rearranged allele. Nucleotide sequence analysis of both alleles reveals that the breakpoint on chromosome 17 is 103 bp from the common *Bgl*I site (Fig. 2). The PK probe, originating from the transposed sequences present in clone λ125 (Fig. 2), was hybridized *in situ* and localized to the q23–q24 bands of chromosome 15 (data not shown), establishing that the cloned rearranged allele contains the t(15;17) translocation breakpoint. Altogether, these data indicate that, in this patient, the promoter region and the first exon of the *RARα* gene have been replaced by a DNA fragment originating from chromosome 15 as a consequence of the translocation.

To determine whether the same type of genomic alteration was encountered in other t(15;17)-positive APL patients, leukaemic DNA samples from eight subjects[13] were analysed by Southern blotting. Hybridization of the *Eco*RI-restricted DNA to the R5 genomic probe, specific for chromosome 17 (Fig. 2), revealed a rearrangement in three out of eight patients, but not in the normal or non-APL leukaemic controls (Fig. 3*a*). When these DNA digests were rehybridized to the PK probe,

FIG. 2 Comparative restriction maps of the normal and rearranged loci. Partial *Mbo*I digests of genomic DNA from NB4 cells were cloned into the λ vector EMBL 3 using standard procedures. The library was screened with a retinoic acid receptor α cDNA probe specific for exon 2 (see Fig. 1*b*). Three (λ143, λ445, λ491) and two (λ164, λ125) positive overlapping clones corresponding respectively to 27 kb of germline and 21 kb of rearranged alleles were analysed. Open bar, sequences from chromosome 17; solid bar, sequences from chromosome 15. The presence of the retinoic acid receptor α exons 1 and 2 are indicated by horizontal arrows. A detailed restriction map of the precise breakpoint (indicated by a vertical arrow) and the corresponding unrearranged locus are shown as well as the partial nucleotide sequence of the two loci. The 300-bp *Bgl*I–*Sma*I and the 450-bp *Bgl*I fragments corresponding respectively to the germ-line and to the translocated alleles encompassing the precise breakpoint region were cloned into M13 vectors and sequenced. The 5-kb *Eco*RI fragment derived from chromosome 17 in the normal allele (R5 probe) and the 0.6-kb *Pst*I–*Kpn*I fragment specific for chromosome 15 in the rearranged locus (PK probe) are indicated. Restriction sites are: R, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; X, *Xho*I; K, *Kpn*I; P, *Pst*I; Bg, *Bgl*I; Pu, *Pvu*II; S, *Sma*I.


λ143
λ445
λ491
R5 probe
normal allele
B B X K B H B R B K R X B K B R R H X K
exon 2
exon 1
1kb
K Bg P P Pu Bg S R S R
CCTGCTCTGCCCTTTGAACCCAGAGCTCGATGCCCC
CCTGCTCTGCCCTTTGAACCCAGTGCTCAGTGTGTG
K Bg P P Pu Bg Bg Bg P K
1 kb
rearranged allele
K B H B R B K K H K K B K R H K
exon 2
PK probe
λ164
λ125

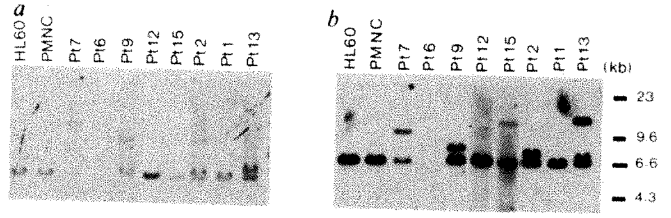
derived from chromosome 15, four out of seven APL patients exhibited a DNA rearrangement (Fig. 3*b*). A germline hybridization pattern with both probes was found in two patients only. The data are summarized in Fig. 3*c*. For patient 9, the extra band for both probes comigrates (Fig. 3*a* and *b*), strongly suggesting that this restriction fragment contains the t(15;17) breakpoint. These results indicate that both loci are frequently rearranged in APL. Assuming that these genomic alterations correspond to the t(15;17) translocations, the data show that the chromosomal breakpoints are tightly clustered.

As the t(15;17) translocation in NB4 deletes the promoter sequences in the rearranged *RARα* gene, we speculated that the chromosome 15-derived region might contain a gene that could control *RARα* transcription. When the PK probe was hybridized to *Taq*I-restricted DNA from yeast, cow, mouse, chicken and human under conditions of relaxed stringency, specific bands appeared in the DNA of vertebrates but not of yeast, suggesting that this probe contains an exon (data not shown). To search for potential transcripts, a PK-M13-derived single-stranded probe (Fig. 4*a*) was hybridized to poly(A)$^+$ mRNA purified from several human haematopoietic cell lines. Five major mRNA species, ranging from 1.8 to 12 kb, were detected (Fig. 4*a*), demonstrating the presence of an exon in the PK probe. It should be noted that this locus, for which we propose the name *myl* for myelocytic leukaemia, is transcribed in the same direction as *RARα* on the translocated allele. To localize the exon, the PK probe was sequenced and computer analysis (PREDICTOR program, ref. 16) showed that a 144-bp region, flanked by canonical splice acceptor and donor sequences, had a very high probability of being an exon (data not shown). Comparison with a protein data bank failed to demonstrate any significant similarity, suggesting that *myl* is a previously undescribed gene.

When *myl* gene expression was studied in NB4 cells, an abundant abnormal transcript of 4.6 kb (Fig. 4*a*), which comigrates with the abnormal *RARα* mRNA (Fig. 1*a*), was detected, suggesting the presence of a cotranscript between the two genes. The 4.6-kb transcript was not present in non-APL leukaemic cells. To substantiate the hypothesis that the t(15;17) translocation generates a *myl/RARα* chimaeric transcript, we performed a reverse transcription/polymerase chain reaction on NB4 and HL60 mRNAs, using primers derived from *RARα* exon 2 and from the 144-bp exon of *myl* (primers 01, 02 and 03 in Fig. 4*b*). When the amplification products were analysed by gel electrophoresis, a sharp band of ~450 bp was observed in NB4 but not in the HL60 negative control. This band hybridized by Southern blotting to the *myl*- and *RARα*-derived 04 and 05 probes respectively (Fig. 4*c*), indicating that the *myl/RARα* junction was amplified in this experiment. These data establish that, in the NB4 cellline, the t(15;17) translocation generates a *myl/RARα* chimaeric transcript.

It has been suggested that altered nuclear receptors may be implicated in oncogenesis[17]. The oncogenic derivative of the thyroid-hormone receptor, v-*erbA*, for example, acts as a constitutive repressor of thyroid hormone-responsive genes[18-19]. On this basis, it was proposed that the v-*erbA* product competes with the wild-type receptor for binding to hormone-responsive elements and exerts its transforming effects by blocking thyroid-hormone-mediated erythrocytic differentiation.

As retinoic acid exerts profound effects on differentiation and proliferation of both non-neoplastic and malignant cells[1-2], alteration of retinoic acid receptors might be involved in oncogenesis. The *RARβ* gene has been shown to be rearranged in a human hepatocellular carcinoma as a result of insertion of hepatitis B virus sequences. Expression of the fusion gene was not documented, however[20]. Our demonstration of a frequent



c

	RARα (R5)	*myl* (PK)
Pt 1	–	–
Pt 2	–	+
Pt 6	+	–
Pt 7	+	+
Pt 9	+	+
Pt 10	+	NT
Pt 12	–	–
Pt 13	–	+
Pt 15	–	+

FIG. 3 Genomic abnormalities in APL patients. *a* and *b*, Control human DNAs from HL60 and PMNC were run next to genomic DNA of patient 7 (Pt7) and 7 other APL patients (Pts 1, 2, 6, 9, 12, 13, 15) harbouring the specific t(15;17) translocation. DNAs were digested by *Eco*RI and hybridized to the chromosome 17-derived R5 probe (*a*). The filters were subsequently rehybridized to the chromosome 15-derived PK probe (*b*). *c*, DNA rearrangements detected in APL patients with either the R5 or PK probe, corresponding to the retinoic acid receptor α (RARα) and *myl* loci respectively. The presence (+) or absence (–), of genomic alterations are summarized. NT, not tested.

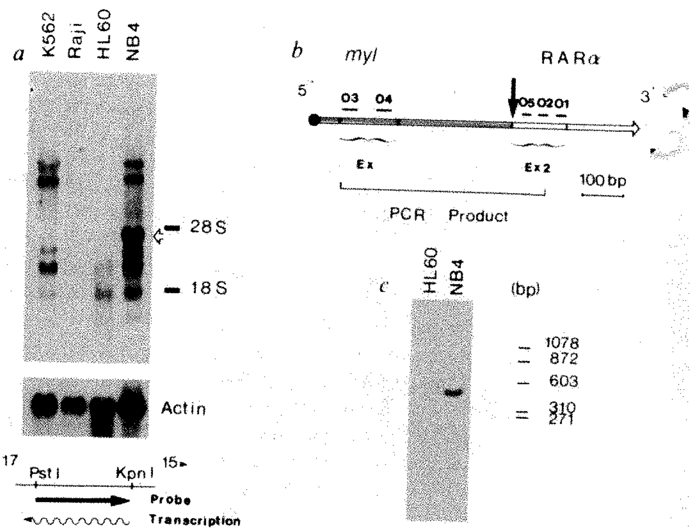


FIG. 4 Analysis of *myl* transcription. *a*, Expression study of the *myl* locus. Poly(A)$^+$ RNA from different human haematopoietic cell lines (K562, Raji, HL60) and from the APL-derived cell line NB4 was blotted onto a nylon filter in 0.05 N NaOH and hybridized to the PK single-stranded DNA probe. The 600-bp *Pst*I–*Kpn*I fragment derived from chromosome 15 was subcloned into M13mp18 to generate a high specific activity ($>10^9$ c.p.m./μg^{-1}) single-stranded probe. The orientation of the single-stranded probe is indicated as well as the deduced sense of transcription. The 4.6-kb abnormal *myl* transcript in NB4 RNA is indicated by an open arrow. The filters were subsequently hybridized to a β-actin probe as a quantitative control. *b*, Schematic description of the reverse transcriptase/polymerase chain reaction (PCR) approach which was performed as described in ref. 26. Five 20-mer oligonucleotides were synthetized for priming and hybridization: 01, 02 and 05 located within retinoic acid receptor α (RARα) exon 2 (Ex2) complementary to the +-strand at positions 553, 443 and 406 respectively[5], 03 and 04 within the presumed exon of the *myl* locus (Ex) positioned respectively 72 and 161 bp downstream from the *Kpn*I site. Poly(A)$^+$ mRNA (1 μg) from NB4 or HL60 cells was reverse transcribed using 01. The cDNA was then subjected to PCR after addition of 02 and 03 as primers. The open bar indicates sequences from chromosome 17-RARα locus and the horizontal striped-bar represents sequences from the chromosome 15-*myl* locus. The sense of transcription is denoted 5' to 3'. The arrow corresponds to the presumed fusion point between the two transcripts. *c*, Southern blot analysis of the amplification products obtained after PCR from cDNAs for NB4 and HL60 RNAs. Probes were oligonucleotides 04 or 05 radiolabelled at the 5' end using [γ-^{32}P]ATP and T4 kinase, to a specific activity of 8×10^6 c.p.m. pmol^{-1}. Both experiments gave similar results and one hybridization only is shown. The DNA markers are aligned.

involvement of the *RAR*α gene in APL provides the first direct link between an alteration of a nuclear receptor and a specific human cancer. One can hypothesize that such rearrangement led to the production of a retinoic acid receptor α whose N-terminal region was deleted and substituted by *myl*-derived sequences. It has been shown that the corresponding domain of the steroid hormone receptors may be involved in both transactivation and target gene specificity[21–23]. The availability of a retinoic acid responsive element[11] should allow assessment of the transactivating properties of the *myl*/*RAR*α fusion protein. In particular, it will be possible to determine whether or not the altered retinoic acid receptor behaves as a dominant negative mutant that might block the expression of retinoic acid target genes presumably involved in granulocytic differentiation.

In this context, it will be most interesting to clarify the apparent paradox between the differentiating effect of retinoic acid in APL and the proposed leukaemogenic role of the α retinoic acid receptor in this disease. ☐

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An essential role for a phospholipid transfer protein in yeast Golgi function

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PROGRESSION of proteins through the secretory pathway of eukaryotic cells involves a continuous rearrangement of macromolecular structures made up of proteins and phospholipids. The protein SEC14p is essential for transport of proteins from the yeast Golgi complex[1]. Independent characterization of the *SEC14* gene[2] and the *PIT1* gene[3], which encodes a phosphatidylinositol/phosphatidylcholine transfer protein in yeast, indicated that these two genes are identical. Phospholipid transfer proteins are a class of cytosolic proteins that are ubiquitous among eukaryotic cells and are distinguished by their ability to catalyse the exchange of phospholipids between membranes *in vitro*[4]. We show here that the *SEC14* and *PIT1* genes are indeed identical and that the growth phenotype of a *sec14-1*^{ts} mutant extends to the inability of its transfer protein to effect phospholipid transfer *in vitro*[4,5]. These results therefore establish for the first time an *in vivo* function for a phospholipid transfer protein, namely a role in the compartment-specific stimulation of protein secretion.

The phosphatidylinositol/phosphatidylcholine (PtdIns/PtdCh) transfer protein from *Saccharomyces cerevisiae* has been purified and partial amino-acid and nucleotide sequences for the protein and the 5′ end of its corresponding structural gene (*PIT1*), respectively, have been determined[3]. We noted that the N-terminal 30 residues of the purified PtdIns transfer protein were identical to the 30 residues of the SEC14p (ref. 2) that were inferred to follow the initiator methionine on the basis of nucleotide sequence data. Moreover, the essential *PIT1* gene had an intron with the same position and sequence as the *SEC14* intron, and encoded a cytosolic polypeptide of relative molecular mass 35,000. These data strongly suggested that *SEC14* was allelic to *PIT1*.

The identity between SEC14p and the PtdIns transfer protein was unambiguously confirmed by a combination of genetic and molecular criteria. These experiments were facilitated by the isolation of *sec14*^{ts} mutants[1] which have been characterized as the conversion of residue 266 of the SEC14p primary translation product from Gly to Asp (ref. 6). Genetic evidence in support of the *SEC14*/*PIT1* allelism was obtained by complementation and nucleotide sequence analysis. Strain CTY1-1A (*MATa*, *ura3-52*, *sec14-1*^{ts}) was transformed with a yeast *PIT1* centrometric plasmid carrying *URA3* as a selectable marker. Transformants to Ura$^+$ prototrophy were selected and tested for growth at 37 °C, a restrictive temperature for *sec14*^{ts} mutants. The *PIT1* gene in single copy restored wild-type growth to strain CTY1-1A. Thus, SEC14p and the PtdIns transfer protein were functionally identical. We confirmed that they were the same protein by completing the *PIT1* nucleotide sequence and showing that it was identical to the *SEC14* gene (not shown). This allelism of *SEC14* and *PIT1* established that *SEC14* is the structural gene for the yeast PtdIns transfer protein.

Our demonstration that *SEC14* is the structural gene for the yeast transfer protein suggested that the primary biochemical defect in *sec14*^{ts} mutants might be the inability of these cells to effect proper mobilization of PtdIns and/or PtdCh under restrictive conditions. To test this prediction directly, cytosolic fractions from isogenic *SEC14* and *sec14-1*^{ts} strains were assayed for both PtdIns and PtdCh transfer activity *in vitro*. The cytosolic fractions from both strains had comparable PtdIns transfer activity when assayed at the permissive temperature of 25 °C (Fig. 1*a*). Although short preincubation of the extracts at 37 °C followed by assay for PtdIns transfer at that same restrictive temperature did not significantly affect the transfer protein activity of the *SEC14* strain, activity in the extract from the *sec14-1*^{ts} strain was almost completely abolished under these conditions (Fig. 1*b*). Moreover, this transfer protein defect was completely reversed when a *CEN4-PIT1* plasmid was introduced into the *sec14-1*^{ts} strain (not shown). We also noted that, in agreement with the reversibility of the *sec14-1*^{ts} defect *in vivo*[1], loss of PtdIns transfer activity in extracts from the *sec14-1*^{ts} strain preincubated and assayed at 37 °C was partially restored when assayed at 25 °C (Fig. 1*c*). Also, extracts prepared from the *sec14-1*^{ts} strain grown for several hours at the restrictive temperature contained significant transfer protein activity when

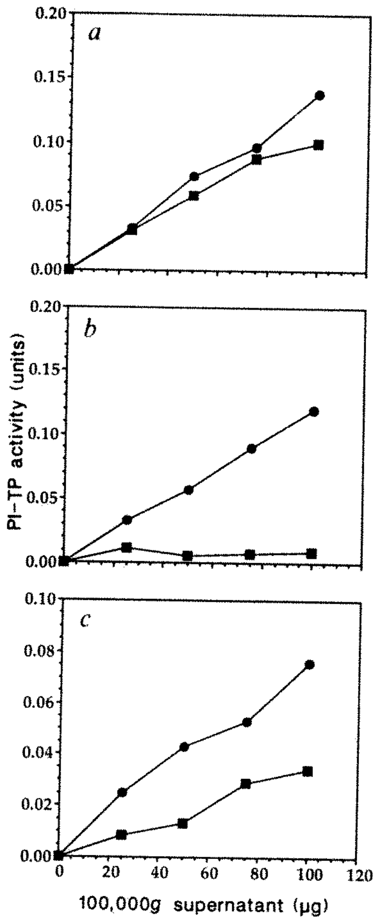

FIG. 1 PtdIns transfer protein (PI-TP) activity in the cytosol of wild-type (*SEC14*, ●) and *sec14-1*^{ts} (■) yeast strains.

METHODS. The isogenic yeast strains CTY182 (*MATa, ura3-52, Δhis3-200, lys2-801, SEC14*) and CTY1-1A (*MATa, ura3-52, Δhis3-200, lys2-801, sec14-1*^{ts}) were grown at 30 °C in 200 ml minimal medium (6.7 g l^{-1} yeast nitrogen base, 20 g l^{-1} glucose, 20 mg l^{-1} histidine and uracil, 30 mg l^{-1} lysine) to an absorbance at 600 nm of 1–1.5. The 100,000*g* supernatant fraction was prepared from each strain and assayed for PtdIns transfer as previously described[3], with the following modifications. Assay mixtures containing phospholipid vesicles, buffer and the 100,000*g* supernatant fraction were either assayed directly at 25 °C (*a*) by addition of [^{3}H]PtdIns-labelled microsomes, or preincubated at 37 °C for 15 min (*b* and *c*) before addition of [^{3}H]PtdIns-labelled microsomes. Assays were carried out at 25 °C (*a* and *c*) or at 37 °C (*b*) for 30 min.

assayed at 25 °C but not at 37 °C. Thus, PtdIns transfer measured *in vitro* correlated directly with *sec14-1*^{ts} gene product function *in vivo*. By contrast, no PtdCh transfer activity could be detected in *sec14-1*^{ts} lysates at either 25 °C or 37 °C, even though the extracts from *SEC14* and *sec14-1*^{ts}/pCEN-*PIT1* strains had clearly measurable PtdCh transfer activity at both temperatures. The lack of each type of transfer activity at the nonpermissive temperature in the *sec14-1*^{ts} strain indicates that PtdIns transfer protein accounts for most of the PtdIns/PtdCh transfer activity in yeast.

What role could a phospholipid transfer protein have in the transport of intracellular protein? Existing evidence points to an execution point for the SEC14p late in the protein maturation pathway in the Golgi complex[1,7,8]. It has been suggested that transfer proteins may be involved in lipid retrieval from the Golgi complex back to the endoplasmic reticulum thereby maintaining an optimal phospholipid/protein ratio for Golgi function[9]. Enzymes such as protein kinase C (ref. 10) and phospholipase A_2 (ref. 11) are dependent on the surface concentration and physical state of membrane components, so dilution by phospholipid might compromise the activity of membrane components of the compartment-specific secretion machinery. The exaggeration of the yeast Golgi complex in *sec14-1*^{ts} mutants[1], and the rapid onset of the corresponding secretory block[2] are consistent with this view.

An alternative hypothesis is that the SEC14p delivers PtdIns, a major acidic phospholipid in yeast synthesized in the endoplasmic reticulum[12], to late Golgi membranes for a specific function. Protein export from *Escherichia coli*[13,14] and protein import into mitochondria[15] depend on acidic phospholipids. The movement of PtdIns to the Golgi at the expense of PtdCh catalysed by the SEC14p could provide the acidic phospholipid content necessary to the catalysis of vesicle-mediated exit of proteins from the Golgi. Alternatively, the Golgi PtdIns may be the mediator of a lipid-mediated signalling pathway which serves to regulate the metabolism of transport vesicles at the level of the late Golgi membranes, in direct analogy to the models formulated for the generation of second messengers from the turnover of PtdIns[16].

Finally, the possibility still remains that phospholipid binding and not phospholipid transfer may be more directly related to the function of the SEC14p. This protein may be related to the class of cytosolic factors that mediate the metabolism of bulk carrier vesicles which move between the endoplasmic reticulum and the Golgi[17]. In this case, binding of either PtdIns or PtdCh would regulate the SEC14p-mediated exit of proteins from the Golgi.

We have demonstrated that the PtdIns/PtdCh transfer protein of yeast is essential for the compartment-specific stimulation of a late Golgi secretory process. These results should provide a foundation for a more detailed study of the largely uncharacterized involvement of phospholipids in secretory pathway function. In this regard we have obtained *S. cerevisiae* mutants that bypass the normally essential SEC14p requirement[6]. □

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Novel potential mitotic motor protein encoded by the fission yeast *cut7*$^+$ gene

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THE structure equivalent to higher eukaryotic centrosomes in fission yeast, the nuclear membrane-bound spindle pole body, is inactive during interphase. On transition from G2 to M phase of the cell cycle, the spindle pole body duplicates; the daughter pole bodies seed microtubules which interdigitate to form a short spindle that elongates to span the nucleus at metaphase[1-5]. We have identified two loci which, when mutated, block spindle formation. The predicted product of one of these genes, *cut7*$^+$ (ref. 6), contains an amino-terminal domain similar to the kinesin heavy chain head domain[7,8], indicating that the *cut7*$^+$ product could be a spindle motor. The *cut7*$^+$ gene resembles the *Aspergillus nidulans* putative spindle motor gene *bimC* (ref. 9), both in terms of its organization with a homologous amino-terminal head and no obvious heptad repeats and in the morphology of the mutant phenotype. But we find no similarity between the carboxy termini of these genes, suggesting that either the *cut7*$^+$ gene represents a new class of kinesin genes and that fission yeast may in addition contain a *bimC* homologue, or that the carboxy termini of these mitotic kinesins are not evolutionarily conserved and that the *cut7*$^+$ gene belongs to a subgroup of *bimC*-related kinesins.

We identified two spindle-formation mutants exhibiting essentially identical phenotypes at the restrictive temperature of 36 °C by screening *Schizosaccharomyces pombe* temperature-sensitive (ts) mutant bank[10] by anti-tubulin immunofluorescence microscopy. One maps to the *cut7* locus on chromosome I[6], whereas the other, *ts549*, maps to a different locus on chromosome II (within 1.3 cM of *cut2* (ref. 6 and S. Uzawa, unpublished data)). Instead of forming a normal mitotic spindle (Fig. 1*j*, *k*), these mutants (*cut7-332* and *ts549*), and the originally identified *cut7* allele (*cut7-446*), exhibit a novel V-shaped staining pattern at 36 °C (Fig. 1*b*, *d*). Microtubules end abruptly at the base of each arm of the V whereas at the distal end they seem to taper away. The V is always associated with the chromosomal domain (Fig. 1*l*). This phenotype is very different from that of *cut1* mutants which affect the spindle pole body structure and number[11]. The *ts* lethality of the *cut7-446* mutation is semidominant (heterozygous diploids can grow at 30 °C, a restrictive temperature for haploid *cut7-446* cells, but cannot grow at 36 °C) whereas that in the *ts549* strain is recessive in a heterozygous diploid state (data not shown).

We examined the nature of the *cut7* defect in detail. A temperature shift of a culture of *cut7-446* cells synchronized in cell cycle progression indicates the timing of events during the abortive mitosis (Fig. 2). Cell viability was unaffected during interphase (up to 105 min), but at the time when wild-type cells would normally enter mitosis and before cell division is initiated, it radically decreases. Coincidentally, cytoplasmic microtubules,

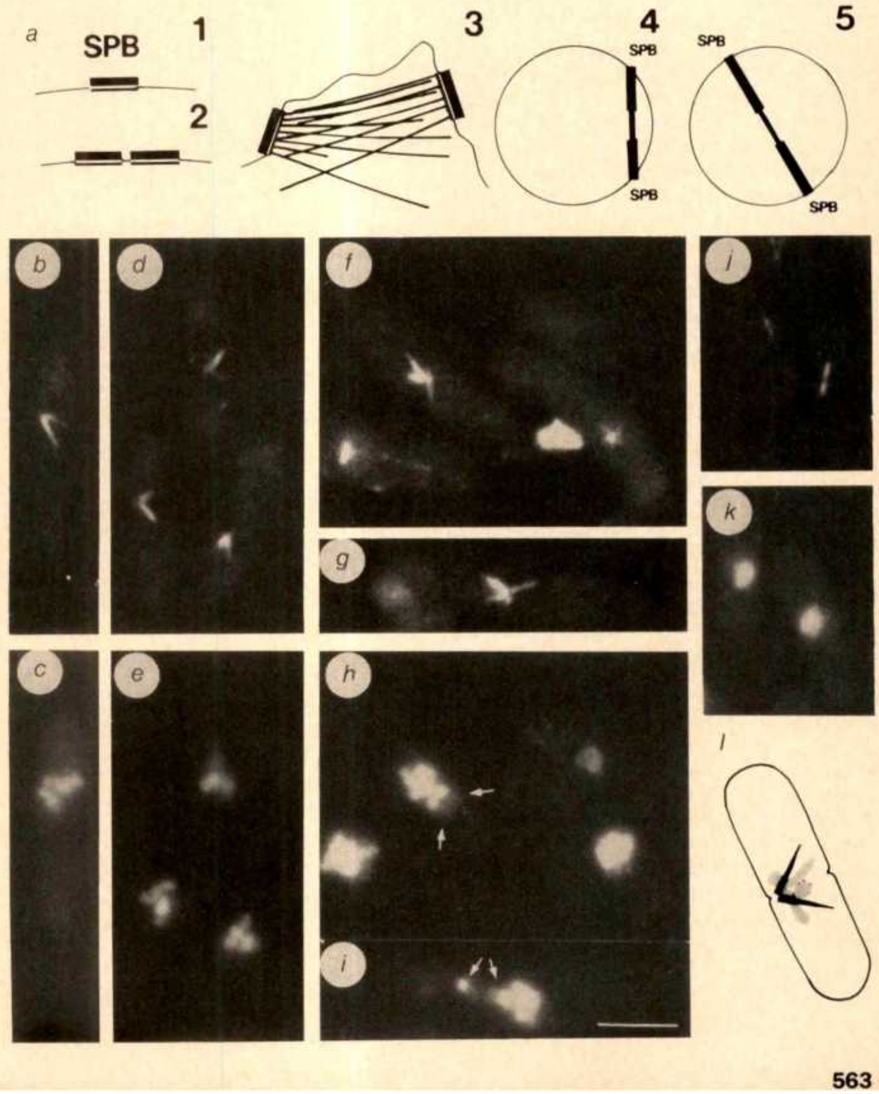

FIG. 1 Immunofluorescence microscopy of *cut7* and *ts549* mutants. *a*, A diagram of the events leading to spindle formation in wild-type cells. The initially single interphase spindle pole body (SPB) (1) duplicates on the transition of the G2/M boundary (2) and the two daughter SPBs are activated and seed microtubules (3). Microtubule interdigitation and SPB separation occur coincidentally and a short prometaphase spindle (5) forms by extension of the prophase spindle (4). Both ends of the metaphase spindle are slightly broader than the middle, presumably because of pole-to-kinetochore microtubules[4,5]. *b*–*k*, Comparison of mutant and wild-type images of mitotic microtubules. *b*, *d*, *f*, *h*, *j*, Anti-tubulin immunofluorescence; *c*, *e*, *g*, *i*, *k*, Diamidinophenyl indole DNA staining. *b*, *c*, A cell from an asynchronous *ts549* culture after 4.5 h at 36 °C, *d*, *e*, cells of a synchronous *cut7-446* culture 100 min after combined synchronization and shift to 36 °C. *f*, *g* and *h*, *i*, Cells of the same *cut7-446* culture 300 min after the temperature shift. Multiple anti-tubulin staining bars are visible along with two chromosome IIIs (arrows), identified by the two ribosomal gene cluster bars extending into the nucleolus from each small diamidinophenyl indole-staining body[16], suggesting that a second cell cycle is in progress. *j*, *k*, Typical wild-type prophase (upper) and metaphase (lower) spindle; *l*, shows a cartoon of the *cut7*$^-$ and *ts549*$^-$ phenotype. Bar 5 μm. METHODS. Cells were grown in rich YPD medium. Cell cycle synchronization was carried out according to the sucrose density gradient technique of Mitchison[17]. YOL1/34 (ref. 18) was used for immunofluorescence[4].

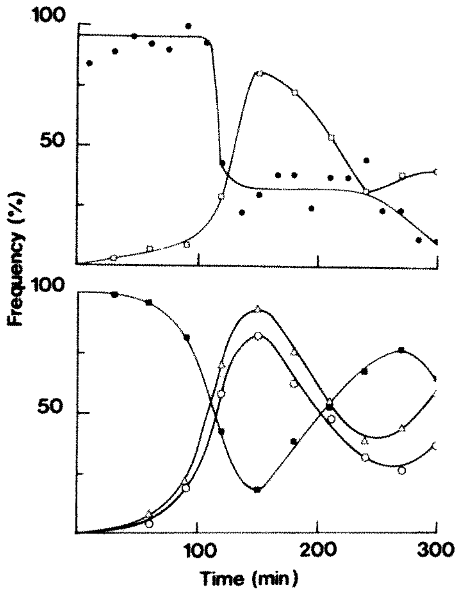

FIG. 2 Quantitative synchronous culture analysis of a *cut7-446* culture a the restrictive temperature. G2 cells were inoculated at time 0 into fresl YPD medium at 36 °C. (Examples of cells from this culture are shown i Fig. 1*d*–*i*). ●, Cell viability; ☐, presence of septa (the division machiner analogous to the cleavage furrows of higher eukaryotes); △, condense chromosomes; ■, interphase microtubules; ○, mitotic microtubules. METHODS. Cells were grown in YPD medium at 25 °C, synchronized[17] anc inoculated into fresh YPD medium at 36 °C at time 0. Cell viability was determined by plating at 25 °C, cell plate index by calcofluor staining[19] chromosome condensation index by diamidinophenyl indole staining[16] anc microtubule configurations by YOL 1/34 anti-tubulin immunofluorescence[4]

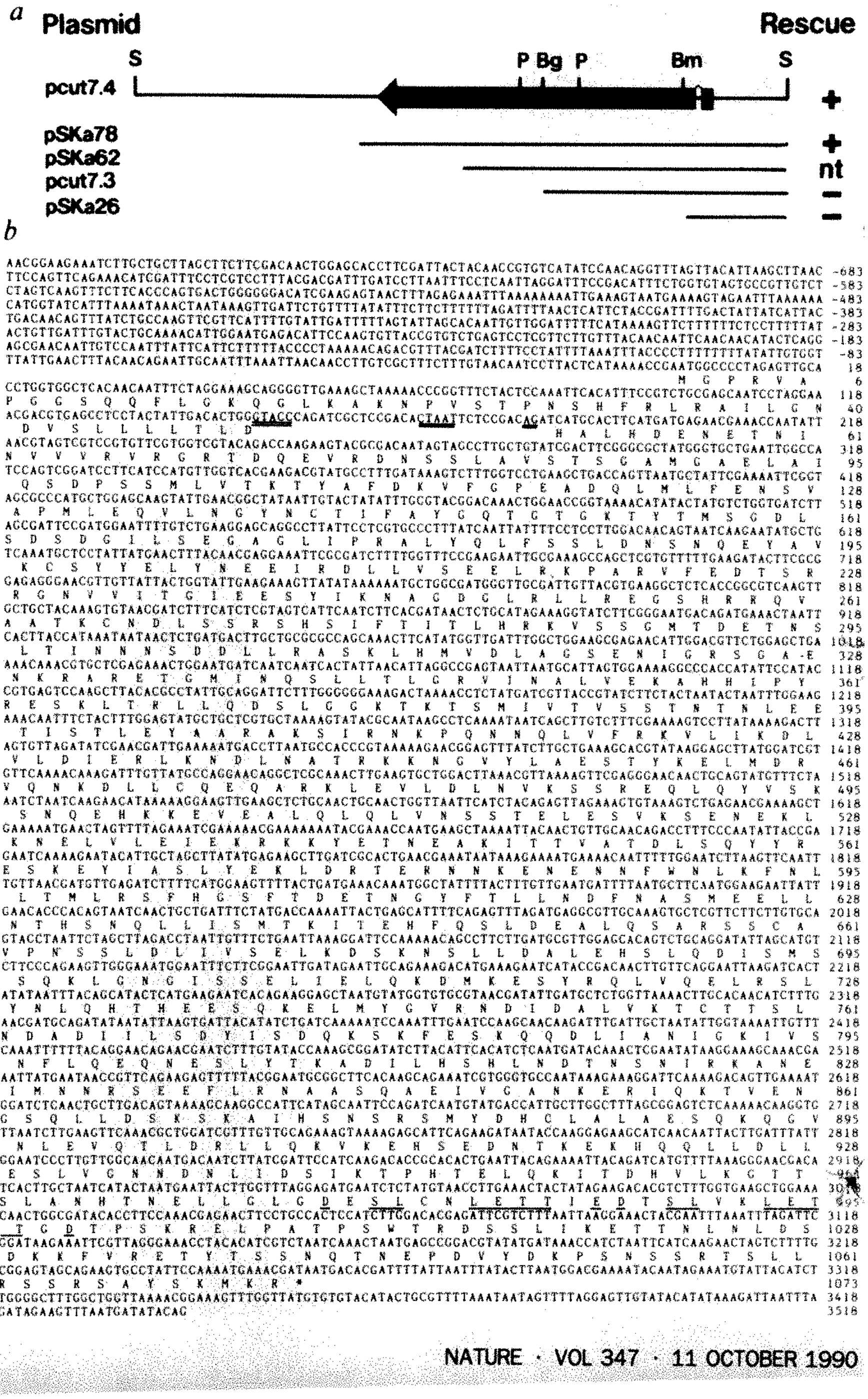

FIG. 3 The nucleotide and predicted amino-acid sequence of the *cut7*$^+$ gene. *a*, Rescue of both *cut7* alleles by subclones of the 7-kilobase (kb) *SacI cut7*$^+$ containing fragment. +, Complementation; nt, no transformants obtained even when transformed into wild type cells; –, no complementation. *b*, The complete nucleotide sequence of the insert SKa78. A 3,260 open reading frame (ORF) encoding a protein of M_r 120,726 starting at –6 with the first ATG at position 1 is interrupted by an intron at nucleotide 368 (consensus sequences are underlined twice). Homology comparisons between *cut7*$^+$ and *bimC* are consistent with the allocation of the intron and ATG. The key residues in a C-terminal direct repeat; DXSLXLETTXE/D single-letter amino-acid notation between positions 975–998 are underlined.

METHODS. *cut7-446* cells were transformed with a genomic DNA bank[20] and assayed for complementation of the temperature-sensitive phenotype. Two unstable transformants out of an estimated 10,000 grew at both 25 and 36 °C. Both contained the same 14-kb insert, which was subcloned to a minimally complementing 7-kb *Sac*1 fragment (*cut7*$^+$.4) which was shown to be continuous in the genome by genomic Southern hybridization. It was shown to contain the *cut7*$^+$ gene by: *NotI*/*LEU2* integration mapping to the correct region of the *cut7*$^+$ containing 1,000-kb chromosome I *NotI* fragment[21]; subsequent tetrad analysis of this strain (PD:NPD:TT = 24:0:0); gene conversion, and the fact that the 7-kb fragment also contains the *his1*$^+$ gene (to be presented elsewhere) which, by classical genetic analysis, maps near to *cut7*$^+$ (ref. 6). *ExoIII* nested sequencing deletions were constructed in Bluescript (Stratagene). Inserts from some of these were subcloned and tested for complementation.

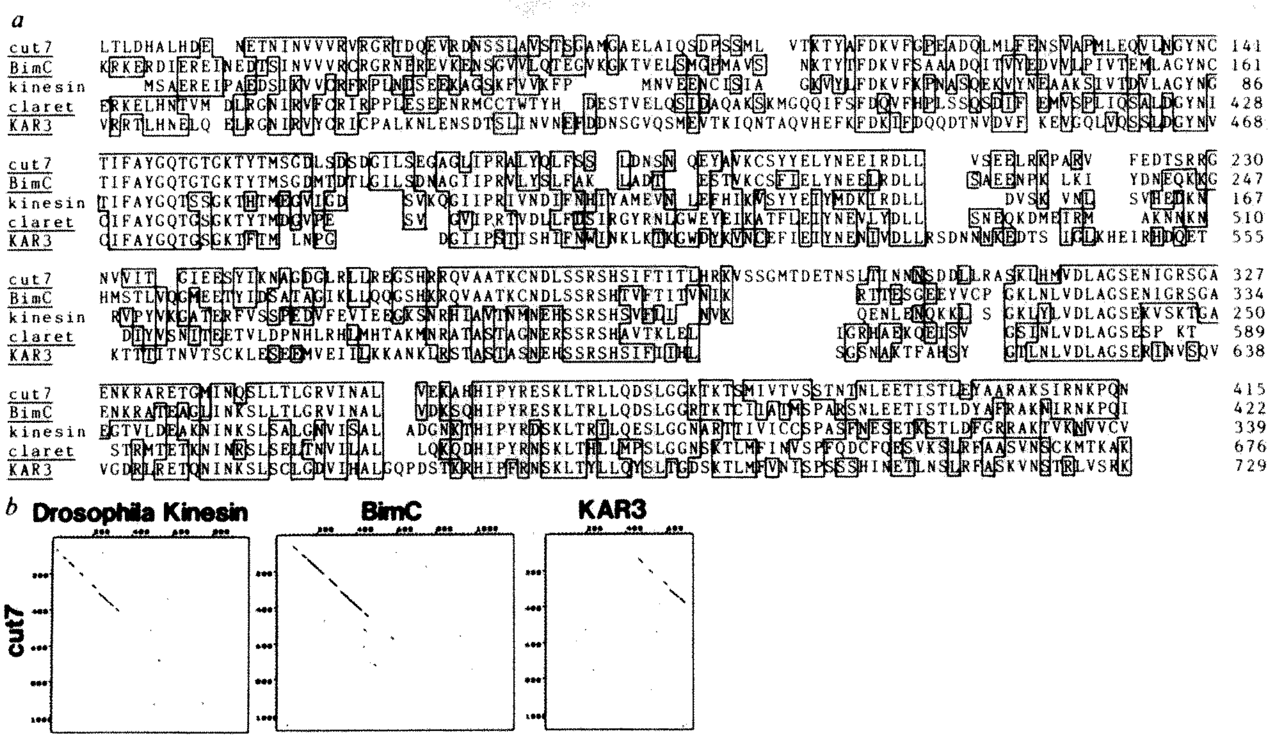
a
cut7 LTLDHALHDE NETNINVVVRVRGRTDQEVRDNSSLAVSTSGAMGAELAIQSDPSSML VTKTYAFDKVFGPEADQLMLFENSVAPMLEQVLNGYNC 141
BimC KRKERDIEREINEDTSINVVVRCRGRNEREVKENSGVVLQTEGVKGKTVELSMGPMAVS NKTYTFDKVFSAAADQITVYEDVVLPIVTEMLAGYNC 161
kinesin MSAEREIPAEDSIKVVCRFRPLNDSEEKAGSKFVVKFP MNVEENCISIA GKVYLFDKVFKPNASQEKVYNEAAKSIVTDVLAGYNG 86
claret ERKELHNTVM DLRGNIRVFCRIRPPLESEENRMCCTWTYH DESTVELQSIDAQAKSKMGQQIFSFDQVFHPLSSQSDIF EMVSPLIQSALDGYNI 428
KAR3 VRRTLHNELQ ELRGNIRVYCRICPALKNLENSDTSLINVNEFDDNSGVQSMEVTKIQNTAQVHEFKFDKIFDQQDTNVDVF KEVGQLVQSSLDGYNV 468
cut7 TIFAYGQTGTGKTYTMSGDLSDSDGILSEGAGLIPRALYQLFSS LDNSN QEYAVKCSYYELYNEEIRDLL VSEELRKPARV FEDTSRRG 230
BimC TIFAYGQTGTGKTYTMSGDMTDTLGILSDNAGIIPRVLYSLFAK LADT ESTVKCSFIELYNEELRDLL SAEENPK LKI YDNEQKKG 247
kinesin TIFAYGQTSSGKTHTMEGVIGD SVKQGIIPRIVNDIFNHIYAMEVN LEFHIKVSYYEIYMDKIRDLL DVSK VNL SVHEDKN 167
claret QIFAYGQTGSGKTYTMDGVPE SV GVIPRTVDLLFDSIRGYRNLGWEYEIKATFLEIYNEVLYDLL SNEQKDMEIRM AKNNKN 510
KAR3 QIFAYGQTGSGKTFTM LNPG DGIIPSTISHIFNWINKLKTKGWDYKVNCEFIEIYNENIVDLLRSDNNNKEDTS IGLKHEIRHDQET 555
cut7 NVVIT GIEESYIKNAGDGLRLLREGSHRRQVAATKCNDLSSRSHSIFTITLHRKVSSGMTDETNSLTINNNSDDLLRASKLHMVDLAGSENIGRSGA 327
BimC HMSTLVQGMEETYIDSATAGIKLLQQGSHKRQVAATKCNDLSSRSHTVFTITVNIK RTTESGEEYVCP GKLNLVDLAGSENIGRSGA 334
kinesin RVPYVKGATERFVSSPEDVFEVIEEGKSNRHIAVTNMNEHSSRSHSVFLI NVK QENLENQKKL S GKLYLVDLAGSEKVSKTGA 250
claret DIYVSNITEETVLDPNHLRHLMHTAKMNRATASTAGNERSSRSHAVTKLEL IGRHAEKQEISV GSINLVDLAGSESP KT 589
KAR3 KTTTITNVTSCKLESEEMVEIILKKANKLRSTASTASNEHSSRSHSIFIIHL SGSNAKTFAHSY GILNLVDLAGSERINVSQV 638
cut7 ENKRARETGMINQSLLTLGRVINAL VEKAHHIPYRESKLTRLLQDSLGGKTKTSMIVTVSSTNTNLEETISTLEYAARAKSIRNKPQN 415
BimC ENKRATEAGLINKSLLTLGRVINAL VDKSQHIPYRESKLTRLLQDSLGGRTKTCIIATMSPARSNLEETISTLDYAFRAKNIRNKPQI 422
kinesin EGTVLDEAKNINKSLSALGNVISAL ADGNKTHIPYRDSKLTRILQESLGGNARTTIVICCSPASFNESETKSTLDFGRRAKTVKNVVCV 339
claret STRMTETKNINRSLSELTNVILAL LQKQDHIPYRNSKLTHLLMPSLGGNSKTLMFINVSPFQDCFQESVKSLRFAASVNSCKMTKAK 676
KAR3 VGDRLRETQNINKSLSCLGDVIHALGQPDSTKRHIPFRNSKLTYLLQYSLTGDSKTLMFVNISPSSSHINETLNSLRFASKVNSTRLVSRK 729
b
Drosophila Kinesin
BimC
KAR3
cut7

FIG. 4 A homology comparison of the *cut7*$^+$ predicted gene product with those of the *Aspergillus nidulans bimC*, budding yeast *KAR3* and *Drosophila melanogaster* kinesin heavy chain and *claret*nd genes. *a*, An alignment of the *cut7*$^+$, *bimC*, *KAR3*, *Drosophila* kinesin and *claret*nd predicted amino-acid sequences. The names and position relative to the first residue are indicated on the left and right side of each sequence respectively. Over the regions shown, *cut7*$^+$ and *bimC* are most homologous with 57% identity. *b*, Harr plots comparing the *cut7*$^+$ amino-acid sequence with those of *Drosophila* kinesin heavy chain, *BimC* and *KAR3* as indicated. The window for comparison is >8 identical residues in 20 continuous amino acids.

markers of the interphase state, disappear and are replaced by the typical *cut7* V staining pattern and not by the normal prometaphase spindle (Fig. 1*j*, *k*). On further incubation, chromosomes decondense and cells containing interphase microtubules reappear, peaking around 270 min, indicating that cell-cycle progression is not blocked. This conclusion is confirmed by the appearance after 300 min at 36 °C of large cells with two sets of chromosomes and multiple anti-tubulin staining bars (Fig. 1*f*–*i*).

Given the *cut7*$^+$ sequence homology data presented below, a plausible interpretation of the mutant phenotype is that spindle pole body duplication is occurring with each entry into mitosis but that the seeded microtubules are unable to interdigitate and subsequently slide apart to form the spindle (Fig. 1*a*, step 3). Instead, the microtubules extend away from the spindle pole bodies at the base of the V and nuclear separation is arrested.

We isolated a single genomic DNA that could rescue the *ts* lethality of both the *cut7-446* and *cut7-332* alleles (but not *ts549*) and which encodes the *cut7*$^+$ gene (as shown by homologous integration). Nucleotide sequence determination of the minimally complementing subclone, pSKa78 (Fig. 3*a*) reveals a large coding region, interrupted by one intron, encoding a 1,073-residue protein (predicted relative molecular mass 120,726 (M_r 21K); Fig. 3*b*) which shares structural similarity with *Drosophila* and squid kinesin heavy chains (Fig. 4*b*)[7,8]. Consistent with the semidominant nature of the *cut7-446* defect, a truncated version of the gene on a high copy number plasmid is toxic to wild-type cells (Fig. 3*a*). We are now constructing deletion strains to investigate the phenotype of the null mutant.

The N-terminal 415 amino acids of the predicted *cut7*$^+$ product, including a potential ATPase site, share 39% identity with the 339 N-terminal 'globular head' domain residues of *Drosophila melanogaster* kinesin heavy chain[7] (Fig. 4*a*, *b*), suggesting that the *cut7*$^+$ gene product could be a microtubule-based ATP-dependent translocator. Following this there is a stretch extending from position 415 to 945 containing only one proline at position 663. This region does not contain an obvious heptad repeat motif.

The *cut7*$^+$ globular head domain has a similar sequence to all the identified kinesin heavy chain-related genes (the highest identity, 57%, being with the *bimC* gene product) (Fig. 4*a*). Although the gene organization of *cut7*$^+$ resembles that of squid and *Drosophila* kinesin heavy chains[7,8] and the *Aspergillus nidulans* mitotic gene *bimC* (ref. 9), it is different from the smaller homologues, *KAR3* (ref. 12) and *claret*nd (refs. 13, 14), in which the motor domain is C-terminal (Fig. 4*b*).

The similarity between *cut7*$^+$ and *bimC* extends beyond sequence comparisons, gene organization and the fact that of the kinesin homologues only these two do not contain a central heptad repeat motif (as a mutation in the *bimC* gene blocks spindle function in a similar way to *cut7* and *ts549* mutations with duplicated spindle pole bodies lying at the base of extending microtubule arrays). Although these similarities could suggest a similar function for the *cut7*$^+$ and *bimC* gene products, the extreme C termini of these genes (a region of kinesin known to interact with other proteins[7,15]) share no significant homology (Fig. 4*b*). This is in contrast to a comparison between *Drosophila* and squid kinesins, where extensive similarity between the C termini has been reported[8], raising the possibility that *cut7*$^+$ and *bimC* may not be functionally interchangeable. As such, the *cut7*$^+$ gene product may represent a new class of kinesin-related proteins to add to the four described to date: kinesin, *KAR3*, *bimC* and *claret*nd. If true, this conclusion suggests that there may be a genuine *bimC* gene in *S. pombe* in a manner analogous to the tubulin gene family, and that the phenotype described here can be indicative of a 'motorless' mutant. Alternatively, the *cut7*$^+$ and *bimC* genes may represent isotypes of the same mitotic kinesin-related molecule, which has not been subject to strong evolutionary conservation. ☐

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Formation of amyloid-like fibrils in COS cells overexpressing part of the Alzheimer amyloid protein precursor

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A PATHOLOGICAL hallmark of Alzheimer's disease is the deposition of amyloid fibrils in the brain. The principal component of the amyloid fibril is β/A4 protein[1,2], which is derived from a large membrane-bound glycoprotein, Alzheimer amyloid protein precursor (APP)[3]. Although the deposition of amyloid is thought to result from the aberrant processing of APP, the detailed molecular mechanisms of amyloidogenesis remain unclear. A C-terminal fragment of APP which spans the β/A4 and cytoplasmic domains has a tendency to self-aggregate[4,5]. In an attempt to establish a cultured-cell model for amyloid fibril formation, we have transfected COS-1 cells with complementary DNA encoding the C-terminal 100 residues of APP. In the perinuclear regions of a small population of DNA-transfected cells, we observed inclusion-like deposits which showed a strong immunohistochemical reaction towards an anti-C-terminal APP antibody or an anti-β/A4 amyloid core-specific antibody. Electron microscope observations of the inclusion-carrying cells revealed an accumulation of amyloid-like fibrils of 8–22 nm diameter near and on the nuclear membrane. The fibrils showed a beaded or helical structure, and reacted positively with the anti-C-terminus antibody by immunoelectron microscopy. These results suggest that the formation of amyloid fibrils is an inherent characteristic of the C-terminal peptide of APP. The present system provides a suitable model for the molecular dissection of the process of brain amyloidogenesis.

We made two cDNA constructs for the expression of APP-C100 (Fig. 1*a*); hβ was designed for expressing only the exact region of APP-C100, and shβ for expressing the APP-C100 region plus a signal peptide for secretion and membrane integration. The ATG codon flanking the 5′-end of the β/A4 region in hβ (corresponding to position 596 of APP695) was used as an initiation codon to synthesize the subsequent 99 amino acids of the C-terminal peptide of APP (APP-C100), whereas the translation initiation codon in shβ corresponds to that of rat preproenkephalin A cDNA[6]. These hybrid cDNAs were inserted into the vector p91023(B), a highly efficient expression vector having an SV40 origin and an adenovirus promoter[7], and transfected into COS-1 cells (African green monkey kidney cells). Transient expression of APP-C100 in the COS-1 cells was examined by immunoblotting and immunoprecipitation methods using an anti-C-terminal antibody raised against residues 681–695 of APP[8] (Fig. 1*b* and *c*). On SDS-polyacrylamide gels, cell extracts of both hβ- and shβ-transfected COS-1 cells contained a major band corresponding to APP-C100 with an apparent relative molecular mass of 16,000 (M_r 16K); the level in shβ-transfected cells was about fivefold higher than that of hβ-transfected cells (Fig. 1*b*). A band of APP-C100 with M_r 16K was also detected in the conditioned medium of shβ-transfected cells but not of hβ-transfected cells (Fig. 1*c*), indicating the efficacy of the signal peptide of the shβ-directed protein and the intracellular containment of APP-C100 in hβ-transfected cells.

We then carried out immunocytochemical staining of the DNA-transfected COS-1 cells with the anti-C-terminal antibody (Fig. 2). About 20% of the hβ-transfected cells were clearly immunoreactive. Unexpectedly, 10–20 μm diameter inclusion-like deposits were found in perinuclear regions of a subpopulation of these immunoreactive cells (4% of total cells) (Fig. 2*a*). The inclusion-carrying cells often showed deformation of the nucleus, suggesting a possible noxious effect of the inclusion on this organelle. Similarly, about 20% of shβ-transfected COS-1 cells were positively stained with the anti-C-terminus antibody. The reticular distribution of the immunoreactivity in shβ-transfected cells suggested the successful integration of expressed APP-C100 into membranes (Fig. 2*b*). Only ~1% of the shβ-transfected cells contained the inclusion, however; this may be because the secretion and membrane integration of APP-C100 in the shβ-transfected cells resulted in reduced cytoplasmic accumulation. The inclusion-like structures in hβ-transfected cells also reacted with an antibody against synthetic β/A4 peptide (residues 1–24) (Fig. 2*c*), which has been shown to react specifically with amyloid cores of senile plaques[8].

We then characterized the intracellular inclusions in hβ-transfected COS-1 cells by electron microscopy. In a single selected cell carrying an inclusion-like deposit, there was an accumulation of filamentous structures having varying diameters (8–22 nm); a beaded or helical structure of these filaments is apparent (Fig. 3*a* and *b*). The deposition of fibrils near and on the nuclear membrane was observed, and rods with a hollow structure were occasionally visible (Fig. 3*c*). These structures resemble amyloid fibrils in the brain amyloid[9–13] and amyloid-like fibrils generated *in vitro* with a synthetic peptide consisting of residues 1–28 of β/A4 protein[14], although in these cases the fibrils are 4–11 nm in diameter. However, the amyloid-like fibrils in hβ-transfected cells are likely to contain the C-terminal region of APP in addition to β/A4 peptide as the anti-C-terminus antibody reacted with the amyloid-like structures near and on the nuclear membrane (Fig. 3*d*). Thus, it is likely that the C-terminal sequence of APP-C100 remains undeleted in most, if not all, the amyloid-like fibrils.

APP may be cleaved in the interior of the β/A4 domain under physiological conditions[15,16] to produce secretory forms of APP, and an abnormal or alternative cleavage pattern must give rise to the amyloid formation in Alzheimer's disease. The present finding indicates that a single abnormal cleavage of intact APP near the N terminus of the β/A4 protein domain, which would generate an N-terminal truncated peptide similar to APP-C100, could potentially form amyloid-like fibrils as long as the amyloidogenic β/A4 sequence remains intact. This raises the possibility that the proteolytic cleavage to release the β/A4 peptide (M_r ~4K) from APP may occur at a later stage in amyloidogenesis after the formation of fibrous structures.

The deposition of senile plaque amyloid occurs in the extracellular space of the brain in Alzheimer's disease or related dis-

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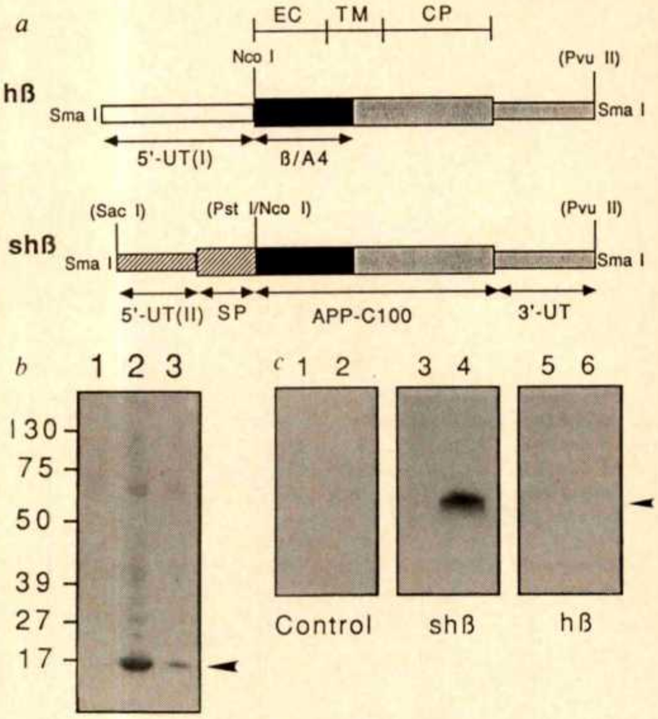

FIG. 1 *a*, Constructs for expression of the C-terminal 100 residues of APP (APP-C100). The coding region (enlarged bar) of APP-C100 consists of extracellular (EC), transmembrane (TM), and cytoplasmic (CP) domains of APP. The derivations of sequences are: 5′-UT(I), 5′-untranslated region of rabbit slow muscle Ca^{2+}-ATPase cDNA; 5′-UT(II), 5′-untranslated region of rat preproenkephalin cDNA; 3′-UT, 3′-untranslated region of human APP; SP, signal peptide encoded in rat preproenkephalin cDNA. The β/A4 domain is shown as a solid bar. Also indicated are the restriction sites used in constructing the hybrid sequences. The restriction sites in parentheses are the original sites converted to other restriction sites for ligation. *b*, Immunoblotting of APP-C100 expressed in hβ- and shβ-transfected COS-1 cells. APP-C100 expressed in DNA-transfected COS-1 cells was analysed by immunoblotting. Lane 1, mock transfected cells; lane 2, cells transfected with shβ; lanes 3, cells transfected with hβ. *M*, size markers (in *M*) at left. Arrowhead, 16K. *c*, Immunoprecipitation of APP-C100 secreted into the conditioned medium of hβ- and shβ-transfected COS-1 cultures. Proteins synthesized in the DNA-transfected cells were labelled with [^{35}S]methionine. APP-C100 secreted into the culture medium was immunoprecipitated with anti-C-terminal antibody. Panels: Control, mock-transfected cells; shβ, cells transfected with shβ; hβ, cells transfected with hβ. Lanes 1, 3, and 5, samples precipitated with preimmune serum; lanes 2, 4, and 6, samples precipitated with anti-C-terminal peptide of APP. Arrowhead, 16K. The value of 16K for immunoreactive APP-C100 (*b* and *c*) is larger than the calculated size (11.3K).

METHODS. *a*, APP-C100 cDNA and its flanking 3′-untranslated region was excised as an *Eco*RI–*Pvu*II fragment of clone B2.3 (ref. 22) and cloned into *Eco*RI/*Sma*I sites of Bluescript SK+(Stratagene); the *Eco*RI–*Pvu*II fragment corresponds to positions 1,796–2,219 of APP695 cDNA[3]. *Nco*I site containing the initiation ATG codon was constructed at the 5′-end of the *Eco*RI–*Pvu*II fragment by replacing 5′-GATAAGCTTGATATCGAATTC-3′ with 5′-GATACCATGGATGCAGAATTC-3′ by oligonucleotide-directed site-specific mutagenesis[23]. The *Bam*HI site, which adjoins the ligated *Pvu*II/*Sma*I site in the multiple cloning sites of Bluescript, was converted to a *Sma*I site by adding *Sma*I linker to the blunted end. 0.4-kilobase (kb) of *Nco*I–*Sma*I fragment, which contains the coding region of APP-C100 and 3′-untranslated region, was then excised. *Sma*I–*Nco*I fragment (positions −215–2) of rabbit slow muscle Ca^{2+}-ATPase cDNA[24] (gift from D. H. MacLennan, University of Toronto) (5′-UT(I)) was ligated to *Nco*I–*Sma*I fragment (hβ). To add a signal sequence (SP) to APP-C100, a 183-bp *Sac*I-*Pst*I fragment of rat preproenkephalin cDNA[6] was ligated to 5′-end of *Nco*I–*Sma*I fragment after blunting both *Pst*I and *Nco*I sites (shβ). An in-frame arrangement of the ligated site was confirmed by the dideoxy-chain termination method[25]. The *Sac*I site at the 5′-end of 5′-UT(II) was blunted and converted to a *Sma*I site by linker attachment. The unique cloning site of *Eco*RI in the sequence of expression vector p91023(B) (ref. 7, gift from R. J. Kaufman, Genetics Institute, Boston) was converted to a *Sma*I site by adding *Sma*I linker to the blunted end. These constructs of hβ and shβ, having *Sma*I sites at both ends, were inserted into the p91023(B) vector at a newly constructed *Sma*I site of the vector. *b*, COS-1 cells were maintained in DMEM supplemented with nonessential amino acids, L-glutamine, antibiotics, and 10% FCS under 5% CO_2/95% air at 37 °C. DNA was transfected into COS-1 cells according to the DEAE-dextran method[26] followed by chloroquine-shock treatment under the conditions described previously[27]. The cells were incubated for 72 h, collected and lysed in buffer containing 0.65 M Tris-HCl (pH 6.5), 2% SDS, 10% glycerol and 5% 2-mercaptoethanol. Samples were then electrophoresed on SDS-polyacrylamide gels (15% acrylamide)[28] and immunoblotted with an antibody against the C-terminal (residues 681–695) of APP695 (1:100)[8], followed by detection using the avidin–biotin–peroxidase complex technique with a kit and the procedure recommended by Vector Labs; peroxidase activity was visualized by reaction with 4-chloro-1-naphthol. *c*, DNA-transfected cells were incubated for 48 h, and the medium was replaced with serum- and methionine-free medium. Proteins synthesized were labelled for 3 h by adding [^{35}S]methionine (Amersham) at 250 μCi ml^{-1} of culture medium. ^{35}S-labelled APP-C100 excreted into the medium was immunopreciptated with anti-C-terminal antibody (1:100) and protein A-bacterial adsorbent (Seikagaku-Kogyo) as described[29]. The precipitate was analysed on SDS-PAGE (15% acrylamide) followed by detection on autoradiography (16-h exposure).

orders. The present cell model of intracellular fibril formation is, however, of great utility for molecular studies of amyloidogenesis. One of the morphological forms of amyloid-like fibrils in the transfected COS-1 cells may be similar to amyloid fibrils *in vivo*. The deposition of amyloid-like structures near and on the nuclear membrane suggests the involvement of membranous structures in the initiation of fibril formation and/or development of the fibrils. Some intracellular proteins may interact with developing fibrils, resulting in modulation or decoration of the filaments. This may account for the variable sizes in the diameter of amyloid-like structures in the hβ-transfected COS-1 cells. In addition, the formation of amyloid-like fibrils in the DNA-transfected cells can be manipulated by various experimental procedures; amyloid-like structures may

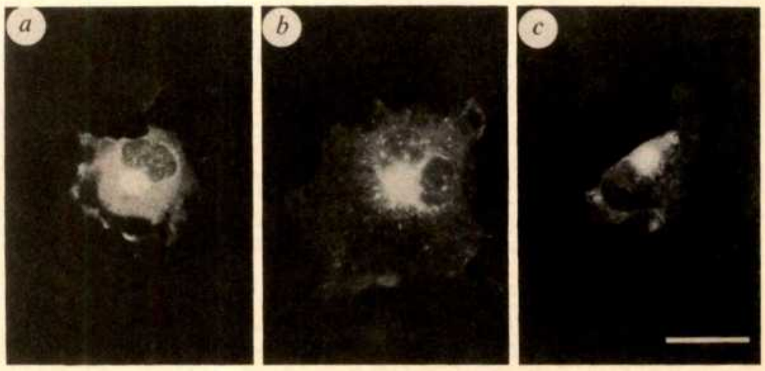

FIG. 2 Fluorescent micrographs of hβ- and shβ-transfected COS-1 cells. DNA-transfected COS-1 cells were cultured for 72 h on gelatin-coated glass coverslips, and then fixed with 90% methanol for 10 min and with methanol-acetone (1:1) for 15 min at −20 °C. Fixed cells were incubated with the anti-C-terminus antibody (1:1,000) or an antibody against synthetic β/A4 protein (1–24) (1:1,000)[8] in TBS (10 mM Tris-HCl, 0.9%NaCl, pH 7.5) for 60 min at 37 °C. After washing with TBS, cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-rabbit immunoglobulins (G+L) (1:80) (Tago) for 30 min at 37 °C. After washing with TBS, immunoreactive cells were visualized and photographed with a fluorescent microscope (Nikon Microphot FXA-FL) using Kodak TMY-400 film. *a*, hβ-transfected cell, stained with anti-C-terminus antibody; *b*, shβ-transfected cell, stained with anti-C-terminus antibody; *c*, hβ-transfected cell, stained with anti-β/A4(1–24) antibody. Note the intensely immunoreactive inclusion-like deposits in the perinuclear region. Scale bar, 50 μm for *a*–*c*.

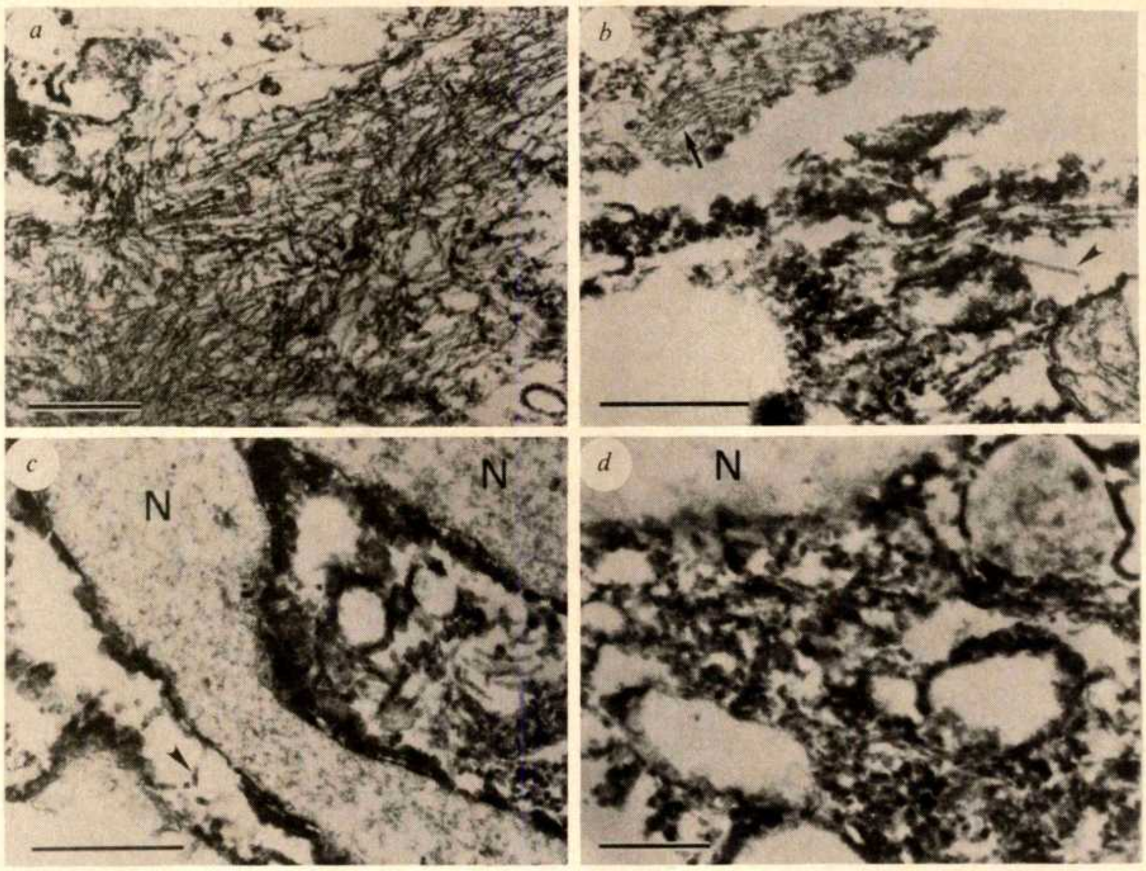

FIG. 3 Electron micrographs of hβ-transfected COS-1 cells having inclusion-like deposits. COS-1 cells transfected with hβ were stained with the anti-C-terminal antibody followed by detection with 3,3′-diaminobenzidine (DAB) peroxidase substrate. Ultrathin sections of selected cells showing intense immunoreactivity were observed under a transmission electron microscope. *a–c*, Sections stained with lead citrate and uranyl acetate. Note the accumulation of amyloid-like fibrils with a relatively homogeneous diameter of 15 nm (*a*) or with varying diameters from 8 nm (*b*, arrowhead) to 22 nm (*b*, arrowhead). These structures were found in the perinuclear region. Parallel rods having an electronlucent centre (*c*, arrowhead) and deposition of fibril-like structures were observed near and on the nuclear membrane (*c*, N). No such structures were found in mock-transfected COS-1 cells. *d*, Unstained section. Fibrils near the nuclear membrane are stained very darkly with the immunoperoxidase reaction products, indicating the presence of the C-terminal sequence in the amyloid-like fibrils. N, nucleus. Scale bars, 500 nm.

METHODS. COS-1 cells were cultured on plastic coverslips and transfected with hβ. Cells were fixed in 1% formaldehyde in PBS for 30 min at room temperature. After washing with PBS, cells were treated with 2% goat serum for blocking, and incubated with the anti-C-terminus antibody (1:1,000) for 1 h at 37 °C. After washing the PBS, biotinylated anti-rabbit IgG and peroxidase-conjugated avidin were added. After fixing with 2.5% glutaraldehyde in PBS, cells were stained using the avidin–biotin–peroxidase technique with the kit and method recommended by Vector Labs. Peroxidase activity was visualized by reaction with 0.05% DAB and 0.01%H_2O_2 for 10 min. DAB-stained samples were postfixed in 2% OsO_4 in PBS for 1 h at room temperature. These samples were dehydrated and embedded in Araldite (Ciba-Geigy). Coverslips were removed after hardening of the resin, and single cells showing intense DAB staining were selected by trimming off the excess resin with a razor blade under a dissection microscope. Ultrathin sections, either unstained or stained with lead citrate and 2% (w/v) aqueous uranyl acetate, were observed under a Hitachi H700H electron microscope at 100 kV.

be modified by cotransfecting cloned cDNAs encoding various kinds of enzymes and other proteins (for example, proteases, protease inhibitors, protein kinases). It may also be feasible to modulate the process of fibril formation by drugs or chemicals. Such studies may provide valuable information on strategies for the prevention of amyloid formation designed to retard the progression of Alzheimer's disease. Furthermore, this COS-1 model presents a suitable system for defining the amino-acid sequences responsible for the formation of amyloid fibrils. Experiments using various DNAs mutated *in vitro* (carrying, for example, site-specific mutations in the β/A4 domain, deletion or substitution of membrane and cytoplasmic domains) can be carried out to examine their amyloidogenic potencies.

Yankner *et al.*[1] have shown that stable transfectants of pheochromocytoma (PC12) cells expressing APP-C100 gradually degenerate when induced to differentiate into neuronal cells by nerve growth factor. Here we have indicated that the dense deposition of amyloid-like fibrils near and on the nuclear membrane might lead to abnormal nuclear function. As it is difficult to estimate the cytotoxic effect of APP-C100 or of amyloid-like fibril formation on the COS-1 cells in the present transient expression system, we are planning to use neural cell lines for examining some pathological effects of intracellular amyloid-like fibrils.

There are several preceding reports on the expression of APP in DNA transfected cells[5,15,17-21]. None of them, however, have succeeded in demonstrating amyloid fibril-like structures. The main reason for our success is the selection of a transient expression system, rather than a stable system, using COS cells and the highly efficient expression vector p91023 in combination. The present transient expression system, which forms amyloid-like structures within 72 h, is an indispensable tool for studying the molecular basis of amyloidogenesis. The resulting information will lead to a better understanding of the pathogenesis of Alzheimer's disease and related disorders.

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Structural and serological evidence for a novel mechanism of antigenic variation in foot-and-mouth disease virus

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CHANGES resulting in altered antigenic properties of viruses nearly always occur on their surface and have been attributed to the substitution of residues directly involved in binding antibody. To investigate the mechanism of antigenic variation in foot-and-mouth disease virus (FMDV), variants that escape neutralization by a monoclonal antibody have been compared crystallographically and serologically with parental virus. FMDVs form one of the four genera of the Picornaviridae. The unenveloped icosahedral shell comprises 60 copies each of four structural proteins VP1–4. Representatives from each of the genera have similar overall structure, but differences in the external features[1–4]. For example, human rhinovirus has a pronounced 'canyon' that is proposed to contain the cell attachment site[1,5–7], whereas elements of the attachment site for FMDV, which involves the G–H loop (residues 134–160) and C-terminus (200–213) of VP1[8,9], are exposed on the surface. Moreover, this G–H loop, which is a major antigenic site of FMDV, forms a prominent, highly accessible protrusion[4], a feature not seen in other picornaviruses. It is this loop that is perturbed in the variant viruses that we have studied. The amino acid mutations characterizing the variants are not at positions directly involved in antibody binding, but result in far-reaching perturbations of the surface structure of the virus. Thus, this virus seems to use a novel escape mechanism whereby an induced conformational change in a major antigenic loop destroys the integrity of the epitope.

A neutralizing monoclonal antibody, 24.31, was raised to the O_1 BFS 1860/67 strain, and serological data indicate that its binding is highly sensitive to virus distortion[10]. By inhibiting the monoclonal antibody binding to virus with peptides (antigen competition) and anti-peptide sera (antibody competition), components of the epitope have been mapped to VP1 regions 146–150 and 200–213, with the critical involvement of residues 146 and either 206 or 207[11]. None of the neutralization-resistant variant viruses selected with this antibody have substitutions within either the G–H loop or at the C-terminus. In fact, the six variants with identified mutations all show substitutions within the B–C loop of VP1; at residues 43, 48 and 59. Neither antibody nor antigen competition assays showed any direct involvement of the B–C loop in binding this antibody. Furthermore, anti-(B–C) loop peptide antibodies failed to recognize native virus, although they did react with virus distorted by

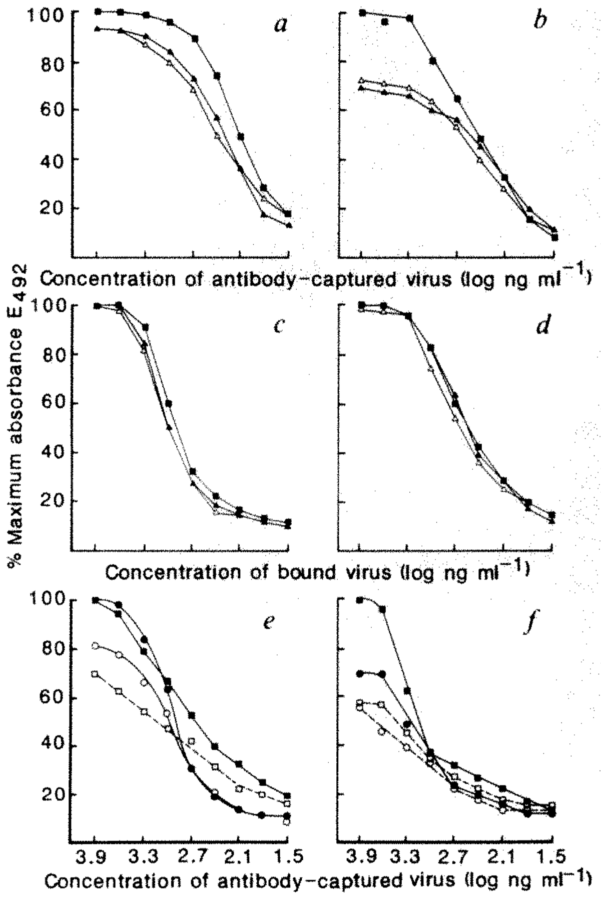

FIG. 1 *a* and *b*, Reactions of parental O_1BFS 1860/67 FMDV (■) and monoclonal antibody escape variants V1 (VP1 Thr 43 → Ala) (△) and V3 (VP1 Thr 43 → Ser) (▲) with anti-virus (*a*) and anti-peptide 141–160 Cys (*b*) antisera in a sandwich ELISA. *c* and *d*, Reactions of the same viruses with antisera to peptides 141–160 Cys (*c*) and 41–60 Cys (*d*) in a direct ELISA. *e* and *f*, Reactions of parental virus (squares) and V4 (circles) (VP1 Ile 48 → Val), serologically indistinguishable from the other variants, with anti-virus (*e*) and anti-peptide 141–160 Cys (*f*) antisera under standard conditions (■, ●) and reducing conditions (□, ○; 0.1% 2-mercaptoethanol) in a sandwich ELISA.

METHODS. In all tests, constant dilutions of antisera were assayed against serial doubling dilutions of virus bound to PVC microplates either with carbonate buffer (direct ELISA) or through an anti-virus IgG capture antibody (sandwich ELISA). The assays were developed using peroxidase-labelled second antibody and peroxide/*o*-phenylenediamine substrate in citrate buffer.

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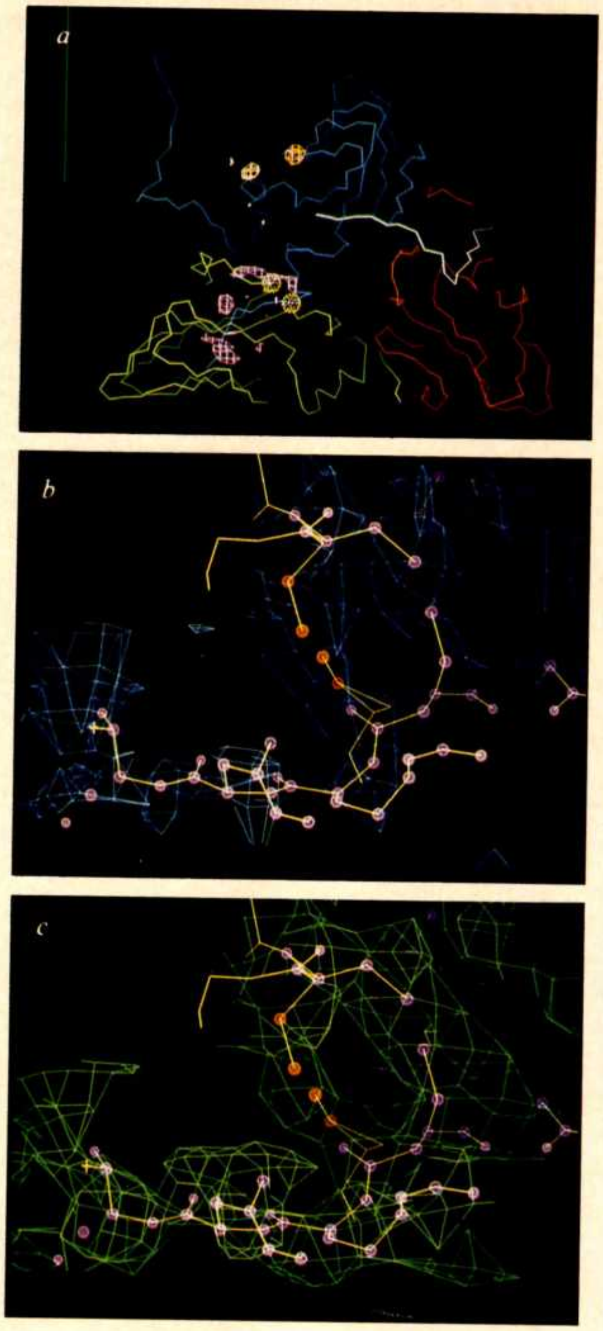

FIG. 2 *a*, An icosahedral subunit of FMDV; the Cα backbone is colour coded: VP1, blue; VP2, green; VP3, red; shown in white is the C terminus of a symmetry-related VP1 (it comes from the five-fold related subunit located to the right). Difference electron density maps for V1 and V3, computed with amplitudes ($F_{\mathrm{obsPAR}} - F_{\mathrm{obsVAR}}$) and $\alpha_{\mathrm{averaged}}$ phases, are superimposed in the region of the B–C loop of VP1. The V1 substitution is identified by a clear negative peak at position 43 of VP1 (shown in orange) consistent with the loss of electron density for the Thr → Ala sequence change. The V2 substitution, a positive peak, corresponds to a gain in electron density at position 59 of VP1 (shown in lemon). This His → Tyr substitution was subsequently confirmed by sequencing. The maps are very clean in the vicinity of these features, reflecting the lack of associated conformational change in the B–C loop region of VP1. Positive difference electron density for V1 is also shown (in pink) corresponding to the switch in population of the disulphide (cysteines marked by yellow, dotted spheres) between position 134 of VP1 and 130 of VP2 with the concomitant movement of strand β-H of VP2 and greater order in the G–H loop of VP1, extending beyond residue 134 in a direction towards the icosahedral threefold axis. The V1 map is representative of the V2 map in this region although of somewhat better quality as the data for V2 are less complete. The difference electron density is contoured at a level of roughly half the maximum peak height in the vicinity of the B–C loop but at a lower level in the region around the G-H loop. Data beyond 4 Å are excluded on account of the error in the weakly measured high angle data; comparable maps result with attenuation of the high angle data. Comparison between these scalar difference maps and vector difference maps[26] showed the scalar maps to be more reliable in this case. *b* and *c*, Fourier syntheses (with amplitudes ($2F_{\mathrm{obs}} - F_{\mathrm{averaged}}$) and the appropriate $\alpha_{\mathrm{averaged}}$ phases) for the parent and variant viruses, respectively, with the V1 model superimposed. The maps show the averaged electron density (corresponding to the five copies of the virus protomer in the I23 asymmetric unit) for both the parent and V1. As we were interested in features on the surface of the virus we performed no solvent flattening. Exactly the same region is depicted in *b* and *c* and the maps are contoured at equivalent levels. The atom positions are represented by pink spheres. Note the switch in the dominant conformation for the S–S bond, linking Cys 134 of VP1 and Cys 130 of VP2, between the parent and V1. Alternative Cys side chain atom positions are shown in red. Beyond this, there is increased order in the electron density for V1 (and likewise V2).

trypsin digestion[12] or by binding to plastic[13,14]. These results suggest that the B–C loop is inaccessible to antibody in the intact O_1 BFS virus.

As the G–H loop had been implicated in the antibody 24.31 epitope, the possibility arose of distinguishing the parent virus from its derived mutants using antisera against G–H loop peptides, even though the viruses have identical sequences in this region. Although the variants reacted as well as the parent virus with anti-virus antisera in sandwich ELISAs (Fig. 1*a*), the results with anti-peptide antiserum (Fig. 1*b*) indicated a reduction in the proportion of antibodies binding to the variants. The distinction was lost when the virus was distorted in a direct binding ELISA (Fig. 1*c*). This finding was corroborated by a 3–4-fold reduction in the ability of the same serum to neutralize the variants compared to the parental strain (data not shown).

These serological data suggest that the substitutions found in the B–C loop are not themselves directly involved in abrogating antibody binding, but induce conformational changes in the G–H loop. To understand the structural basis of these results, we examined two variants V1 (VP1 Thr 43 → Ala) and V2 (VP1 His 59 → Tyr) by X-ray crystallography. The crystals, grown as described previously[15], were isomorphous with those of the parent virus. Crystallographic details are presented in Table 1. In the present study, individual phases were obtained for each of the data sets by further cycles of averaging[16] against the relevant set of structure factors using iterative real-space averaging and solvent flattening from the set of phases obtained previously[4]. Thus, there was no possibility of bias towards any one of the structures. The appropriate phases were used to calculate a variety of electron density maps. To preserve features on the surface of the virus we performed no solvent flattening on these syntheses (for details see Fig. 2).

The difference electron density maps for both variants show clear evidence for mutations in the B–C loop of VP1 as expected (Fig. 2*a*). The maps are clean around the substitutions and show no indication of local conformational changes. But differences in electron density are evident at a considerable distance from the mutations and are similar for both variants. We have previously reported bifurcated density for a disulphide bond linking residues 134 of VP1 and 130 of VP2 in the parent virus[4], which we interpreted as indicating the presence of multiple loop conformations in agreement with the absence of electron density for residues 137–156 of VP1. Of the two conformations, one is predominant in the parent virus, whereas the other predominates in both variants (Fig. 2*b*, *c*). This conformational change results in the concomitant movement of strand H of VP2. Furthermore, there is more ordered density in both variants, extending beyond residue 134 of VP1 towards the icosahedral three-fold axis, away from the B–C loop. The 137–142 amino acid sequence of VP1 correlates well with this density and has been built into the electron density map (Fig. 2*b*, *c*). Residues 136–139 of VP1 lie on top of strand C at the edge of the β-barrel of VP2 (Fig. 3), forming an extra strand of β sheet and lying in a position

TABLE 1 Summary of X-ray data

	Parent	V1	V2
Number filmpacks processed	60	46	12
% Completeness to 2.9 Å	69.9	70.4	31.8
RI (all data included)	14.1	10.3	14.9
R	17.84	14.91	16.35
C	0.92	0.94	0.93

All three data sets were derived from a substantial number of crystals of cloned virus. Further, as there is no significant proteolytic degradation of the virus in the crystals[15], we are confident that they contain homogeneous populations of intact particles. The crystals belonged to the cubic space group I23, $a = 345$ Å, with fivefold non-crystallographic redundancy. Data were collected on small angle oscillation photographs with the exception of some supplementary low angle data for V1 collected on the Enraf Nonius FAST electronic area detector. The SERC Synchrotron radiation facility at Daresbury, UK was used throughout, subject to the usual disease security measures. The protocol for the collection of photographic data was as previously described[4] with the implementation of an automatic indexing procedure[25] for analysis of the V2 data. The procedure for the collection and analysis of the FAST data will be described elsewhere.

$$RI = \frac{\sum_h \sum_i |(I_h - I_{hi})|}{\sum_h \sum_i I_{hi}} \times 100$$

$$R = \frac{\sum_h |(|F_{h,\mathrm{obs}}| - |F_{h,\mathrm{calc}}|)|}{\sum_h |F_{h,\mathrm{obs}}|} \times 100$$

$$C = \frac{\sum_h (\langle F_{\mathrm{obs}}\rangle - |F_{h,\mathrm{obs}}|)(\langle F_{\mathrm{calc}}\rangle - |F_{h,\mathrm{calc}}|)}{[\sum_h (\langle F_{\mathrm{obs}}\rangle - |F_{h,\mathrm{obs}}|)^2 \cdot \sum_h (\langle F_{\mathrm{calc}}\rangle - |F_{h,\mathrm{calc}}|)^2]^{1/2}}$$

I_h is the weighted mean measured intensity of the observations I_{hi}. F_{obs} is the observed structure amplitude of reflection h. F_{calc} is the calculated structure amplitude of reflection h as a result of back-transforming the averaged and modified electron density map.

occupied by part of the 'antigenic puff' of VP2 in the other genera of the picornaviruses, as predicted[1].

In the light of the structural differences observed, the antigenic consequences of reducing the VP1-VP2 disulphide bond were investigated. In the presence of 0.1% 2-mercaptoethanol, which does not affect infectivity, the ability of antiserum to the G-H loop peptide to distinguish between the parent and a variant virus was abolished (Fig. 1*e*, *f*). This supports the hypothesis that the disulphide bond between VP1 and VP2 contributes to e antigenic properties of the FMDV loop.

The structural differences provide an explanation of the serogical results, pointing to the conclusion that the G-H loop exists in at least two conformations or families of conformation in these viruses: (1) The 'down' conformation, for which there is good structural evidence in the variants, where the G-H loop extends downwards until it approaches close to the pentamer boundary and the icosahedral three-fold axis; (2) the 'up' conformation (postulated to predominate in the parent), where the G-H loop overlays the B-C loop in the region of residues 43 to 59 of VP1. Although there is no conclusive evidence for the 'up' conformation, very weak protein density is seen in association with the B-C loop, consistent with this hypothesis. This is the conformation recognized by antibody 24.31. In the 'down' conformation of the G-H loop, residue 146, a crucial component of the 24.31 epitope is too distant from the B-C loop for direct involvement of the substituted residues in antibody binding (Fig. 3). It seems that the variants escape neutralization through substituted residues in the B-C loop interacting with the G-H loop to destabilize the 'up' conformation, thus destroying the integrity of the epitope recognized by this antibody.

Of the seven FMDV serotypes only type O has the potential to form a VP1-VP2 disulphide bond involving the G-H loop of VP1. The constraints imposed by this bond may be related to the observation that most monoclonal antibodies to serotype O viruses recognize conformationally 'sensitive' epitopes, whereas a high proportion of monoclonal antibodies to serotype A and C viruses recognize immobilized peptides representing the G-H loop[17,18]. The conformational restriction of the disulphide bond thus seems to drive the loop towards the less intrinsically stable 'up' conformation. The substitutions in the variant viruses seem to overcome this. Even with viruses lacking the disulphide bond, however, indirect modulation of the conformation of the G-H loop may be important as substitutions in the region of VP2 underlying the 'down' conformation of the loop result in changes in antigenicity and cell attachment[19]; properties attributed to the G-H loop itself[8,9,11,12,20-22]. Although the loop remains too disordered to decipher the detailed mechanism of antibody escape, its dynamic properties may have a significant role in the kinetics of antibody binding as only a small change in the free energy of binding will be needed to reduce dramatically the proportion of antibody molecules that attach.

None of the substitutions in the variant viruses occurred within the previously mapped epitopic regions. As these regions are important for viral attachment to cell receptors[8], it is possible that the residues crucial for binding of antibody 24.31 are coincident with those necessary for receptor binding and are, therefore, intolerant of mutation. But mutations within the G-H

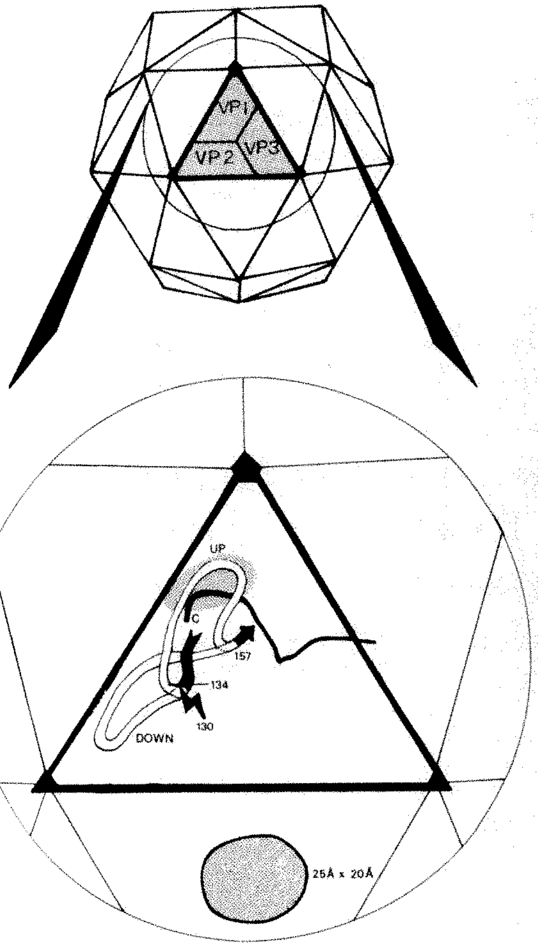

FIG. 3 Schematic diagram of the icosahedral subunit showing the proposed conformational switching of the VP1 G-H loop. The 'down' conformation reflects the extension of the C-terminal end of the FMDV loop as built for V1 running parallel to strand β-C of VP2 before turning down towards the threefold axis and then looping back to the defined location for residue 157. The location of the epitope described involving residues in the G-H loop in the postulated 'up' conformation and the C terminus of VP1 from the adjacent protomer (depicted in black) is stippled. Its location with respect to the disulphide between residue 134 of VP1 and 130 of VP2 can be visualized. An area scaled to represent the approximate area of an antibody 'footprint' is shown.

loop have been selected under pressure from other monoclonal antibodies[22]. It is known that FMDVs vary in their receptor specificity[23], so different conformations of the G–H loop may be recognized by different receptor molecules. Alternatively, the same receptor may induce conformational changes in the G–H loop on binding.

In summary, we present a model of antigenic variation achieved through a switch in the predominant conformation of a surface loop of FMDV. This change is triggered by substitutions of residues in another surface loop, in a mechanism akin to the allosteric switching of enzymes[24]. ☐

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Altered protein conformation on DNA binding by Fos and Jun

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THE protein products of the c-*fos* and c-*jun* proto-oncogenes (Fos and Jun, respectively) form a heterodimeric protein complex that interacts with the activator protein-1 (AP-1) binding site and regulates gene transcription in response to extracellular stimuli[1]. Protein dimerization is mediated primarily by a coiled-coil-like structure termed the leucine-zipper and DNA binding occurs primarily through regions of each protein rich in basic amino acids that contact both strands of the AP-1 site[2–8]. The precise nature of the protein–DNA interaction is unknown as studies concerned with dimerization and DNA binding by Fos and Jun have relied on indirect methods to investigate protein–protein–DNA interactions. Here we have developed assay systems using fluorescence spectroscopy and circular dichroism to monitor dimerization and DNA binding directly. The results indicate that the interaction of Fos and Jun with DNA results in an altered conformation of the protein dimers and an increased α-helical content. These techniques may have general application in studies concerning the interaction of transcriptional regulatory proteins with specific DNA target sequences.

The sensitivity and specificity of fluorescence spectroscopy techniques have many applications in the analysis of biologically important molecular interactions[9]. For example, fluorescently labelled myosin has been used to investigate the interaction of myosin with nucleotides and actin with great precision[10]. We have used a similar approach to develop solution assays for analysing the dimerization and DNA-binding properties of Fos and Jun. The proteins used for these studies contained the leucine-zipper and DNA-binding domains of Fos (amino acids 116–211) and Jun (amino acids 224–334) fused to an N-terminal peptide containing six histidine residues[8]. These proteins were purified from *Escherichia coli* and are active in dimerization, DNA binding and *in vitro* transcription assays[22]. Fluorescently labelled Jun224–334 was prepared using the thiol-specific reagent 5-iodo-acetamido fluorescein (IAF) (Fig. 1*a*). There are two potential target cysteine residues for modification in Jun224–334, one located within the DNA-binding domain and one just C-terminal of the leucine zipper[11]. Under the labelling conditions used, ~1.3 mol of IAF were incorporated per mol of Jun, indicating that each of the Cys residues were labelled. Fluorescently labelled Jun was readily visualized under ultraviolet light after electrophoresis on a sodium dodecyl sulphate polyacrylamide gel (Fig. 1*b*).

Jun exists primarily as a homodimeric protein complex in solution. The addition of Fos results in an exchange of subunits leading to an accumulation of the more stable Fos–Jun heterodimer[12–14]. This exchange process was readily observed using IAF-labelled Jun (Jun*). Excitation of Jun* at 490 nm produced a clear emission spectrum with a maximum at 520 nm, which increased substantially after addition of Fos116–211 (Fig. 2*a*). The increase in fluorescence is likely to be caused by a change in the local microenvironment of the fluorophore. Fluorescein is significantly quenched by acidification and has an increased quantum yield at high pH (ref. 10). Although the emission maximum increased, we did not detect a shift in the wavelength of the excitation or emission spectra. Titration of Jun* with increasing amounts of Fos resulted in a linear elevation of fluorescence (Fig. 2*b*). The half-maximal increase occurred at a ratio of Jun*: Fos of 1:1 and saturating levels were reached at a ratio of ~1:2 (Fig. 2*b*). The presence of fluorescent adducts on each partner in the Jun* homodimer may have caused interference resulting in a reduced level of fluorescence of Jun* homodimers compared with Fos–Jun* heterodimers. To test th possibility, we incubated Jun* in the presence of increasir amounts of unlabelled Jun224–334. A mixture of Jun* an unlabelled Jun exhibited increased fluorescence compared with Jun* homodimers (Fig. 2*c*). As with Fos plus Jun*, the half-maximal increase occurred at a ratio of 1:1. Thus, the exchange of both Jun homodimers and Fos–Jun heterodimers in solution can be monitored by fluorescence spectroscopy.

One of the IAF-labelled Cys residues in Jun224–334 lies within the DNA-binding domain. This is a highly reactive Cys, conserved among all members of the c-*fos* and c-*jun* gene families. Recently, we demonstrated that DNA binding by Fos and Jun is regulated by a reduction/oxidation mechanism at this residue[15]. Therefore, we reasoned that DNA binding might cause a sufficient change in the local environment to affect fluorescence. Gel-shift assays demonstrated that the IAF-labelled Jun was capable of binding to DNA (not shown). The presence of a double-stranded oligonucleotide containing an AP-1 binding site (AP-1) caused a significant increase in the emission maximum of a mixture of Fos–Jun* complexes (Fig. 2*e*). The addition of increasing amounts of the oligonucleotide caused a corresponding increase in fluorescence, although under the conditions used here we did not reach completely saturating levels (Fig. 2*f*). In contrast, incubation of Jun* or Fos plus Jun* with a nonspecific DNA sequence (deoxyinosine/deoxycytidine, poly(dIdC)) or with an oligonucleotide containing an SP-1 site

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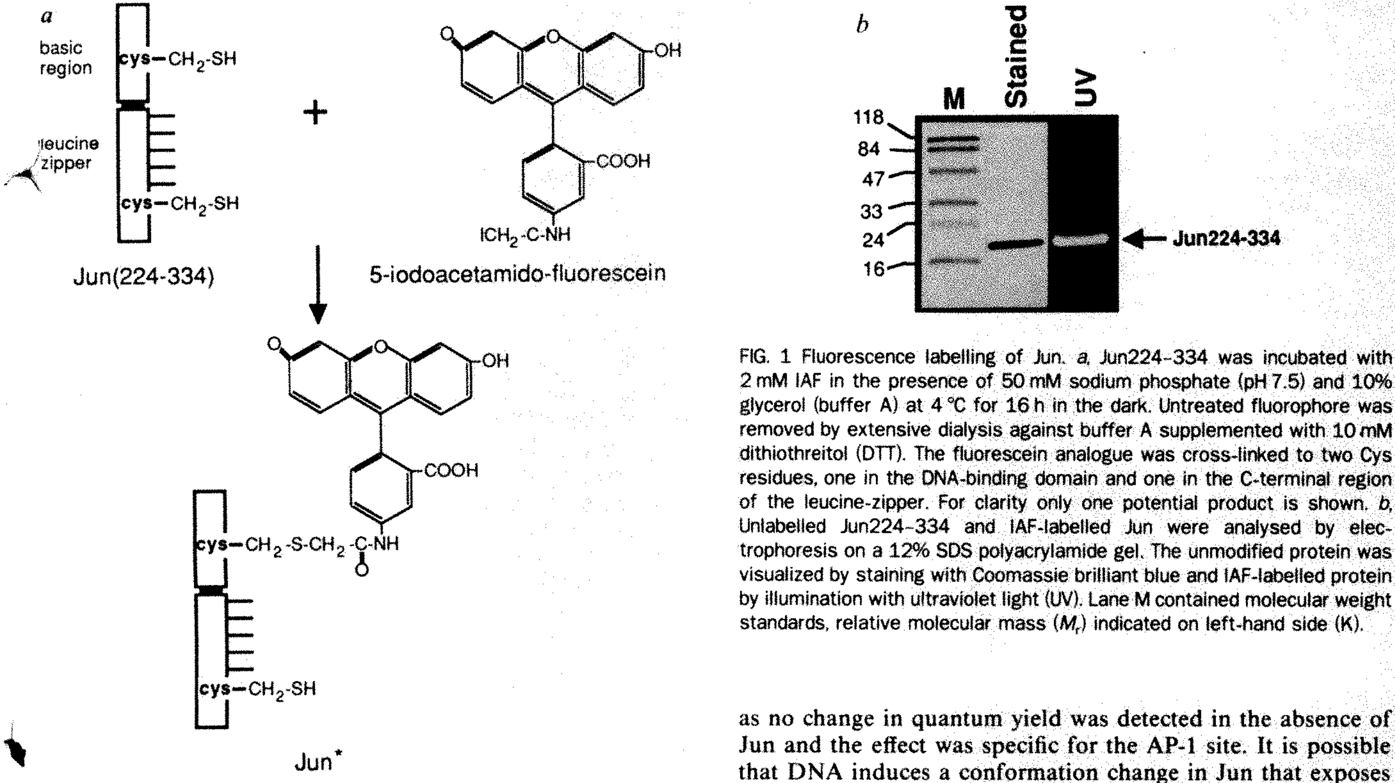
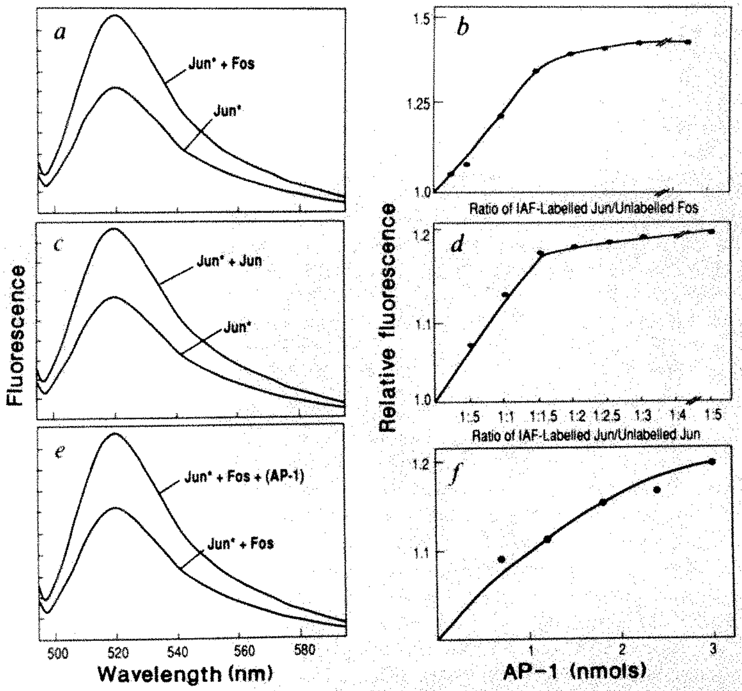


FIG. 1 Fluorescence labelling of Jun. *a*, Jun224-334 was incubated with 2 mM IAF in the presence of 50 mM sodium phosphate (pH 7.5) and 10% glycerol (buffer A) at 4 °C for 16 h in the dark. Untreated fluorophore was removed by extensive dialysis against buffer A supplemented with 10 mM dithiothreitol (DTT). The fluorescein analogue was cross-linked to two Cys residues, one in the DNA-binding domain and one in the C-terminal region of the leucine-zipper. For clarity only one potential product is shown. *b*, Unlabelled Jun224-334 and IAF-labelled Jun were analysed by electrophoresis on a 12% SDS polyacrylamide gel. The unmodified protein was visualized by staining with Coomassie brilliant blue and IAF-labelled protein by illumination with ultraviolet light (UV). Lane M contained molecular weight standards, relative molecular mass (M_r) indicated on left-hand side (K).

had no significant effect (data not shown). We have also analysed mutated Jun proteins that contain serine substituted for the Cys residues[15]. After incubation with IAF, Jun with no Cys was not labelled, Jun with Cys only in the DNA-binding domain was labelled very efficiently and Jun with Cys in the C-terminal region was labelled inefficiently. Thus, it is likely that the modified Jun studied here contains the fluorophore primarily on the highly reactive Cys in the DNA-binding domain. The changes in fluorescence that occurred during dimerization or DNA binding were only seen with the mutated Jun containing the fluorophore in the DNA-binding domain. Thus, DNA binding by Jun* results in a change in the fluorophore microenvironment in the DNA-binding domain although the nature of the change is not clear. It is unlikely that fluorescein binds to DNA as no change in quantum yield was detected in the absence of Jun and the effect was specific for the AP-1 site. It is possible that DNA induces a conformation change in Jun that exposes the fluorophore to the highly basic DNA-binding domain. Mutagenesis studies and experiments with sulphydryl-modifying agents suggest that the Cys in the DNA-binding domain is in close proximity to DNA[15]. We are currently using different fluorophores on each Cys residue in both partners of the heterodimeric complex, and modified bases in the target DNA sequence in an attempt to obtain estimates of the distances between each group in solution.

It has been suggested that the DNA-binding regions of Fos and Jun participate in a 'scissors-grip' interaction with DNA in which α-helical regions lie along the major groove[16]. In this model, it is unlikely that the DNA-binding domains would maintain a stable configuration in the absence of DNA. The DNA-binding domains of Fos and Jun are adjacent to the

FIG. 2 Analysis of dimerization and DNA-binding properties of Fos and Jun by fluorescence spectroscopy. The emission spectrum of 1 μM IAF-labelled Jun (Jun*) in buffer A was recorded at 27 °C between 500–600 nm using a Perkin-Elmer MPF66 fluorometer. The spectra of: *a*, Jun* alone, Jun* plus 5 μM Fos116–211; *c*, Jun* alone, Jun plus 5 μM unlabelled Jun224–334; and *e*, 2 μM Jun* plus 5 μM Fos in the presence and absence of 3 μM of a specific double-stranded oligonucleotide containing an AP-1 site (AP-1) (ref. 12) were determined. The effects of increasing amounts of: *b*, Fos 1–5 μM; *d*, Jun 1–5 μM; and *f*, 1–3 μM of the AP-1 oligonucleotide on the emission maximum of Jun* were also examined.



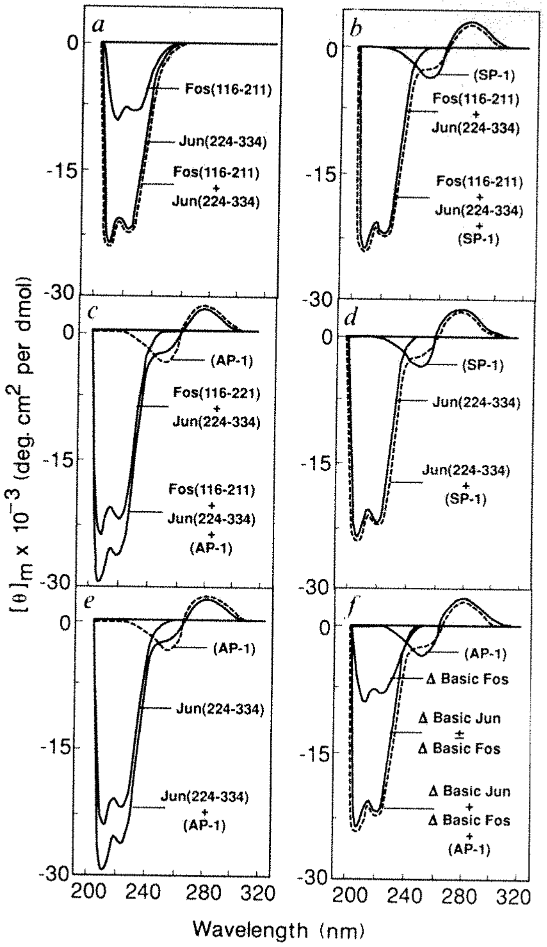


FIG. 3 Effects of dimerization and DNA-binding on CD spectra of Fos and Jun. Polypeptides corresponding to Fos116-211 (Fos) and Jun224-334 (Jun) were analysed in buffer A containing 1 mM DTT at a final protein concentration of 0.2 mg ml^{-1}. (Identical results were obtained using buffer A without glycerol.) Mixtures of proteins and nucleic acids were incubated at 37 °C for 30 min and spectra were recorded for 15 min at 25 °C using a Jasco J500 spectrometer. Representative traces are shown of the spectra of: *a*, Fos, Jun, equimolar mixtures of Fos plus Jun and the reference spectrum of buffer A; *b*, Fos plus Jun in the presence and absence of 0.1 mg ml^{-1} of a double-stranded oligonucleotide containing an SP-1 site (SP-1) and the SP-1 oligonucleotide alone; *c*, Equimolar mixtures of Fos plus Jun in the presence and absence of 0.1 mg/ml^{-1} of a double-stranded oligonucleotide containing an AP-1 site (AP-1) and the AP-1 oligonucleotide alone; *d*, Jun in the presence and absence of 0.1 mg ml^{-1} of an oligonucleotide containing an SP-1 site and the SP-1 oligonucleotide alone; *e*, Jun in the presence and absence of 0.1 mg ml^{-1} of an oligonucleotide containing an AP-1 site and the AP-1 oligonucleotide alone; *f*, analysis of mutated Fos and Jun proteins lacking the DNA-binding region (Δ Basic) in the presence and absence of 0.1 mg ml^{-1} of an oligonucleotide containing the AP-1 site and the AP-1 oligonucleotide alone. Δ Basic Fos is Fos116-211 lacking amino acids 139-144 and Δ Basic Jun is Jun 224-334 lacking amino acids 260-266. These proteins do not bind to DNA[3]. The spectra obtained with Δ Basic Jun alone and a mixture of Δ Basic Jun plus Δ Basic Fos were identical and are referred to as Δ Basic Jun ± Δ Basic Fos. Spectra that overlap are indicated by broken lines. The percentage helicity was calculated as described[21].

leucine-zipper region, which has been proposed to form a stable α helix that participates in a coiled-coil interaction[3,17]. Circular dichroism (CD) and two-dimensional nuclear magnetic resonance studies on synthetic leucine-zipper peptides have supported this contention[14,18]. We analysed the CD spectra of purified Fos116-211 and Jun224-334 to determine their overall α-helical content. Jun224-334 gave a clear spectrum with minima at 208 and 222 nm indicating an α-helical content of ∼50% (Fig. 3*a*). In contrast, Fos116-211 gave a relatively weak spectrum indicating a lower α-helical content of ∼23%. The simplest explanation of these data is that Jun forms homodimers that stabilize the α-helical region of the leucine-zipper; whereas Fos does not. This would predict that in a mixture of Fos plus Jun, which form heterodimers, the α-helical content would be similar to that of Jun homodimers. Indeed, this was the case; equimolar mixtures of Fos plus Jun gave a spectrum identical to that of Jun homodimers (Fig. 3*a*). Thus, Fos has a conformation in a Fos-Jun heterodimer substantially different from that of the free Fos monomer in solution.

To examine the effects of DNA on α-helical content, the CD spectrum of Fos-Jun heterodimers was analysed in the presence of an oligonucleotide containing an SP-1 site (Fig. 3*b*) or AP-1 site (Fig. 3*c*). The presence of the AP-1 site caused a distinct increase in the α-helical content of the protein complex of ∼10%; whereas the SP-1 site had no significant effect (Fig. 3*b*, *c*). Similar observations were obtained with Jun homodimers (Fig. 3*d*, *e*). The effect of the AP-1 site on conformation was concentration-dependent and saturation levels were reached at a 1:1 ratio of protein:DNA. These results indicate that binding of Fos-Jun heterodimers and Jun homodimers to a specific DNA target sequence, the AP-1 site, causes a change in conformation that results in increased α helicity. This effect may be interpreted in two ways: (1) DNA binding stabilizes the conformation of protein dimers resulting in an overall increase in α-helical content, or (2) the DNA-binding domains of Fos and Jun take on an α-helical configuration only in the presence of DNA. We favour the latter interpretation as Jun homodimers are less stable than Fos-Jun heterodimers, yet they have identical CD spectra (Fig. 3*a*). Similarly, CD studies on synthetic leucine-zipper peptides revealed that per cent helicity, unlike the T_m, was not affected by the stability of the dimers[14]. Furthermore, mutated Fos and Jun proteins lacking functional DNA-binding domains (Δ Basic Fos and Δ Basic Jun) failed to show increased α helicity in the presence of the AP-1 site, although Δ Basic Fos showed an increased α helicity when dimerized with Δ Basic Jun (Fig. 3*f*). These data are consistent with the 'scissor-grip' model of DNA binding by proteins containing a leucine-zipper(s)[16]. But they neither confirm nor deny the proposed helix-bend-helix structure.

The finding that the DNA-binding domains of Fos and Jun have different configurations depending on whether or not they are associated with DNA has an interesting implication. This would provide a mechanism whereby non-DNA-bound (inactive) and DNA-bound (active) transcription factor complexes might be distinguished. This could be an important feature [illegible] transcriptional regulation as non-DNA-bound complexes cou[illegible] sequester components of the transcriptional machinery by t[illegible] phenomenon of squelching[19]. In Fos and Jun, the activatio[illegible] regions of each protein which are responsible for transcriptiona[illegible] stimulation are located very close to the DNA-binding domains[20,22]. Thus, the conformation of these regions is likely to be altered depending on the state of the DNA-binding domain (that is, whether or not it is associated with DNA). Thus, conformational changes of transcription factors in the presence of specific DNA-binding sites could represent a novel mechanism for regulation. □

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Folding transition in the DNA-binding domain of GCN4 on specific binding to DNA

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PROTEIN-DNA recognition is often mediated by a small domain containing a recognizable structural motif, such as the helix-turn-helix[1] or the zinc-finger[2]. These motifs are compact structures that dock against the DNA double helix. Another DNA recognition motif, found in a highly conserved family of eukaryotic transcription factors including C/EPB, Fos, Jun and CREB, consists of a coiled-coil dimerization element—the leucine-zipper—and an adjoining basic region which mediates DNA binding[3]. Here we describe circular dichroism and ^{1}H-NMR spectroscopic studies of another family member, the yeast transcriptional activator GCN4[4,5]. The 58-residue DNA-binding domain of GCN4, GCN4-p, exhibits a concentration-dependent α-helical transition, in accord with previous studies of the dimerization properties of an isolated leucine-zipper peptide[6]. The GCN4-p dimer is ~70% helical at 25 °C, implying that the basic region adjacent to the leucine zipper is largely unstructured in the absence of DNA. Strikingly, addition of DNA containing a GCN4 binding site (AP-1 site) increases the α-helix content of GNC4-p to at least 95%. Thus, the basic region acquires substantial α-helical structure when it binds to DNA. A similar folding transition is observed on GCN4-p binding to the related ATF/CREB site, which contains an additional central base pair. The accommodation of DNA target sites of different lengths clearly requires some flexibility in the GCN4 binding domain, despite its high α-helix content. Our results indicate that the GCN4 basic region is significantly unfolded at 25 °C and that its folded, α-helical conformation is stabilized by binding to DNA.

The DNA-binding domain of the yeast transcription factor GCN4, located within the C-terminal 60 residues of the protein[7], can be divided into two subdomains. The C-terminal subdomain (32 residues) contains four leucine residues in a heptad repeat, the leucine zipper. Physicochemical studies of a synthetic leucine-zipper peptide[6,8] and 'zipper swap' experiments[9,10] support the proposal[11] that the leucine repeat forms a hydrophobic dimerization interface. The *N*-terminal subdomain (the basic region; 26 residues) is rich in lysine and arginine residues, many of which are conserved in this family of transcription factors. Domain swap experiments demonstrate that the basic region has the predominant role in making specific DNA contacts[12].

To further our understanding of the structural basis for DNA recognition by this family of transcription factors, we have examined by circular dichroism (CD) and ^{1}H-NMR spectroscopy, the structure of a peptide (GCN4-p; residues 225-281) that includes the basic region and leucine repeat of GCN4. GCN4-p was overexpressed in *Escherischia coli*, and its DNA-binding properties were analysed by gel-retardation assay (Fig. 1). GCN4-p binds to synthetic DNA containing a consensus AP-1 target site with an affinity ($K_{\text{apparent}} = 2 \times 10^{-8}$ M; Fig. 1, lanes 1-6) comparable to that of intact GCN4 (ref. 4). GCN4-p fails to bind to an unrelated DNA sequence (the λ O_L1 operator; Fig. 1, lanes 13 and 14). These results show that the DNA-binding properties of the GCN4-p peptide are very similar to those of the intact GCN4 protein.

At high protein concentrations (>100 μM) the far-ultraviolet CD spectrum of GCN4-p shows the characteristics of α-helical structure (Fig. 2*A*). The calculated α-helix content of GCN4-p under these conditions is 70-73%, or 40-43 residues in helical conformation, calculated on a mean residue ellipticity at 222 nm and 25 °C of $-22{,}700$ deg cm^2 dmol^{-1}. No increase in helix content is seen at higher protein concentrations. Progressive attenuation of the α-helix-associated absorption bands is observed with decreasing peptide concentration (Fig. 2*A*), indicating an unfolding transition that is presumably accompanied by dissociation of the dimer into unstructured monomers. Previous studies of a leucine-zipper peptide (residues 252-281[6]) indicated that it is dimeric throughout the concentration range studied here. We suggest that decreased stability of GCN4-p dimers relative to those of the leucine-zipper peptide may result from repulsion of the basic region subdomains.

The unfolding of GCN4-p with decreasing concentration is also seen by ^{1}H-NMR spectroscopy. The leucine-zipper subdomain contains two aromatic residues, Tyr 265 and His 266, whose resonances are well resolved and serve as intrinsic probes of GCN4-p dimerization (Fig. 2*B*). At high and low peptide concentrations (Fig. 2*B*; spectra *a* and *f*, respectively), the resonance linewidths ($1/T_2$) of these residues are consistent with the presence of dimeric and monomeric species. Higher-order oligomers would be expected to show broader resonances, which are not observed. At intermediate peptide concentrations, the pairs of aromatic NMR resonances corresponding to monomeric and dimeric species have relative intensities that vary with GCN4-p concentration (Fig. 2*B*, spectra *b-e*). By contrast, NMR resonances of basic region residues (for example, Thr 236) show no intensity change over this range of peptide concentration (data not shown). These results unambiguously assign the concentration-dependent increase in α-helix content of GCN4-p Fig. 2*A*) to the leucine-zipper region of the peptide.

Previous studies of the dimerization properties of an isolated leucine-zipper peptide showed that the orientation of α-helices in the dimer is parallel, and it was proposed that the leucine zipper is a coiled-coil structure[6]. Two-dimensional NMR studies of this peptide shows that it forms a continuous α-helix, with symmetrical dimerization contacts that are evident from the single resonance observed for each proton in the peptide[8]. This symmetry is consistent with the parallel association of peptide α helices in a coiled-coil arrangement. The dimer-related protons of GCN4-p also show a single resonance (for example, His 265 and His 265'; Fig. 2*Ba*), suggesting that the dimerization interface of the GCN4-p DNA binding domain is similar to that observed for the leucine-zipper peptide. The estimated lifetime of GCN4-p dimers at 25 °C is between 10 ms and 1 s, on the basis of the exchange properties of NMR resonances assigned to the leucine-zipper region (see Fig. 2). In conjunction with the modest dissociation constant of GCN4-p for DNA, these observations suggest that unfolding and reassembly of GCN4, and other transcription factors utilizing a coiled-coil dimerization interface, may occur without significant kinetic barriers, thereby facilitating subunit exchange. Such exchange is thought to provide a general mechanism for regulating patterns of gene expression.

Our results indicate that the leucine zipper dimerization interface is present in a functional GCN4 DNA-binding domain; but the α-helix content of GCN4-p (40-43 residues) is greater

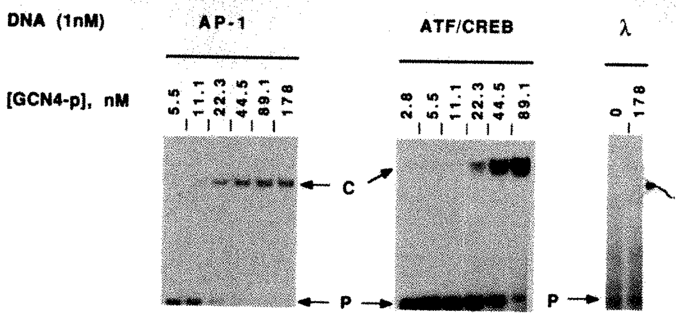

FIG. 1 Gel-retardation assay of GCN4-p binding to oligonucleotides containing the AP-1/TPA-responsive element consensus binding site (5′-GAGATGAGTCATCTC-3′; lanes 1–6), the activating transcription factor/cyclic AMP-responsive element consensus binding site (5′-GAGATGACGTCATCTC; lanes 7–12), or the phage λ O_L1 site (5′-GATACCACTGGCGGTGATATC-3′; lanes 13 and 14). 5′-^{32}P-labelled oligonucleotide (1 nM) was incubated with the indicated concentrations of GCN4-p peptide for 30 min at 23 °C in buffer containing 50 mM KPO_4 (pH 7.5), 50 mM KCl, 100 μg ml^{-1} BSA and 5% glycerol then electrophoresed on a 5% polyacrylamide gel. Following electrophoresis, gels were dried and the free oligonucleotide probe (P) and GCN4-p–DNA complexes (C) were visualized by autoradiography. The GCN4-p peptide (residues 225–281 of the GCN4 protein) was expressed in *E. coli* from a bacteriophage T7 RNA polymerase promoter[24,25] (10–12 mg peptide per litre culture) and purified by a combination of ammonium sulphate precipitation and phosphocellulose chromatography as described elsewhere to >98% homogeneity as judged by SDS-PAGE. An extinction coefficient of 1,313 M^{-1} cm^{-1} at 280 nm, derived by amino acid analysis and ultraviolet spectrophotometry, was used to determine the concentration of GCN4-p monomer in the stock solution. Oligonucleotides used in all assays were synthesized on a Milligen or an Applied Biosystems oligonucleotide synthesizer and purified by HPLC or gel electrophoresis.

than can be accounted for by the leucine-zipper subdomain alone (32–36 residues). Indeed, the CD spectrum of GCN4-p exhibits more α helix as the temperature is lowered. At 1 °C, GCN4-p is ~80% helical, as calculated from a mean residue ellipticity of −27,000 deg cm^2 dmol^{-1} under these conditions (Fig. 2*C*). We propose that the basic region of GCN4-p exists as an ensemble of structures in the absence of DNA, with a substantial population of completely folded molecules present only at low temperature. Support for this proposal comes from studies of a synthetic basic region peptide (GCN4-br; residues 224–249), which exhibits, between 25 °C and 1 °C, a partial α-helical transition similar in magnitude to that of GCN4-p over the same temperature range (Fig. 2*C*). The thermal unfolding transitions of GCN4-p are described in detail elsewhere[13].

The CD spectrum of the specific complex between GCN4-p and a synthetic AP-1 site (21 base pairs) is shown in Fig. 3*A*. A significant increase in the magnitude of the helix-associated bands at 205 and 225 nm is observed for the complex (Fig. 3*A*), relative to GCN4-p alone (Fig. 2*A*). This change is opposite to that expected from the contribution of DNA, which exhibits positive ellipticity in this region of the spectrum (Fig. 2*A*, upper spectrum). The spectral perturbations resulting from complex formation are therefore the result of a DNA-dependent structural transition in the protein, as evidenced by the difference spectrum of the complex minus the sum of its constituents (Fig. 3*A*, Δ). The magnitude of this difference spectrum increases linearly as a function of added AP-1 binding site, reaching saturation at a stoichiometry of two GCN4-p monomers per DNA site (data not shown). At saturation, the DNA-bound conformation of GCN4-p is 95–100% α-helix (Fig. 3*A*). Thus, our results indicate that the folded conformation of the GCN4-p basic region is predominately α-helical, but that the folded state is significantly populated only at low temperature (Fig. 2*C*) or on binding to DNA (Fig. 3*C*). The helical transition observed for the isolated basic region peptide at low temperature (Fig. 2*C*) suggests that these conformational properties of the GCN4

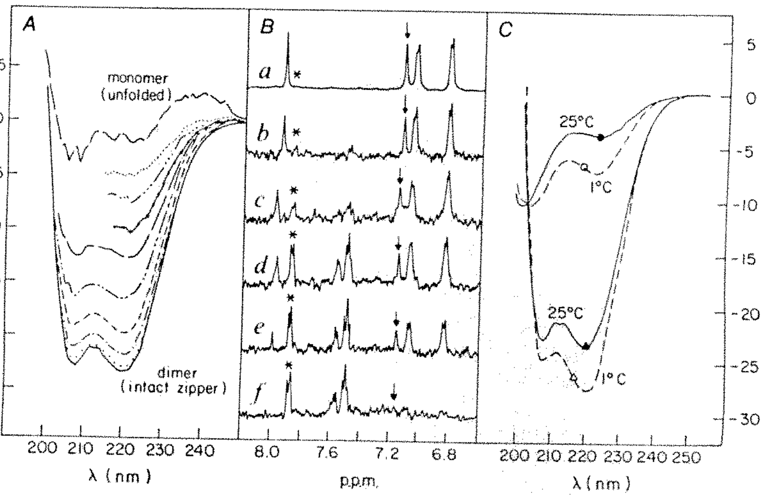

FIG. 2 *A*, normalized far-ultraviolet CD spectra of GCN4-p at different protein concentrations in 200 mM KCl, 50 mM potassium phosphate (pH 7.0 and 25 °C). Protein concentrations (bottom to top, μM): 155, 38, 13, 4.3, 1.6, 1.2, 0.9, 0.6, 0.3, 0.15. Gel-filtration chromatography demonstrates that GCN4-P is dimeric at a protein concentration of 2 mM under these conditions[13]. The GCN4-p monomer seems to be unfolded, as has previously been observed among certain prokaryotic DNA-binding proteins[26]. To maintain adequate signal-to-noise at lower protein concentrations cuvettes with longer path lengths were used (range: 0.5–10 mm). Protein concentration was determined by quantitative amino acid analysis following acid hydrolysis of an aliquot. Mean residue ellipticity [θ] was calculated with calculated molecular weight 6,874 g m^{-1} (118.5 g per mol-residue). *B*, Aromatic region of the 500 Mhz ^{1}H-NMR spectrum of GCN4-p at different protein concentrations in 500 mM KCl, 50 mM potassium phosphate (pD 7.0, direct meter reading) in 99.98% D_2O. Fortuitously, the apparent dimerization constant of GCN4-p is weaker under these conditions, facilitating NMR study. *a–f*, 1,000, 100, 50, 30, 20 and 10 μM, respectively. Dimer-specific and monomer-specific aromatic resonances are observed in slow exchange on the NMR time scale. Arrow indicates dimer-specific Hδ resonance of H266, which decreases in amplitude as the protein concentration is decreased. *, monomer-specific aromatic resonance of Y265, which increases in amplitude as the protein concentration is decreased. The slow-exchange condition provides a lower bound of 10 ms on the lifetime of the dimer. The unresolved resonances of the branched-chain aliphatic sidechains (leucine, isoleucine and valine), which are primarily in the leucine-zipper moiety, are in intermediate exchange under these conditions (data not shown), providing an upper bound of 1 s on the lifetime of the dimer. This short lifetime of the GCN4-p dimer is in apparent conflict with the previous observation suggesting that heterodimers between intact GCN4 and the C-terminal domain were very stable at 0–4 °C in the absence of DNA[5]. But recent experiments suggest that the original observations were artifactual, probably because of co-precipitation of the proteins with excess nonspecific nucleic acid in the particular wheat germ extracts employed at that time. The chemical shift scale was referenced to TSP resonance at 0 p.p.m. *C*, Normalized CD spectra of the GCN4-p dimer (△) and the basic-region peptide (○) at 25 °C and 1 °C in the buffer described in (*A*). The concentration of GCN4-p was 145 μM; the concentration of the basic-region peptide was 154 μM. The sequence of the basic-region peptide is (N)SSDPAALKRARNTEAARRSRARKLQR-$CONH_2$ (single letter amino-acid code). Its structure was verified by amino-acid analysis and sequencing; no truncation or deletion products were observed. The helix content is estimated from the mean residue ellipticity at 222 nm, assuming that for a 100% helical peptide this value is −33,000 deg cm^2 dmol^{-1} at 0 °C, and at higher temperatures is attenuated 0.3% per °C before unfolding[6,27–29] GCN4-p exhibits a cooperative unfolding transition (midpoint 65 °C) similar to that observed in the isolated GCN4 leucine-zipper peptide[27], as will be described elsewhere[13].

basic region are locally determined and are independent of the adjoining leucine-zipper dimerization interface.

Two sets of experiments demonstrate that the folding transition observed on addition of the AP-1 DNA to GCN4-p requires specific complex formation. First, the spectral changes do not occur at high ionic strength (Fig. 3*A*). The CD spectrum of the GCN4-p/AP-1 mixture in 1.5 M KCl is nearly identical to that of GCN4-p alone. The CD spectrum of GCN4-p alone is unaltered in 50–1,500 mM KCl (not shown). Moreover, the KCl-dependence of the DNA-induced spectral changes is consistent with the elution properties of AP-1 site-containing oligonucleotides from a GCN4 affinity column[14]. Second, the CD spectrum of a mixture of GCN4-p with a heterologous DNA control element that does not bind to GCN4-p (the λ O_L1 operator site, Fig. 1) resembles the sum of the spectra of the constituents alone (Fig. 3*B*). The calculated difference spectrum for this mixture (Fig. 3*B*, Δ) has less than 10% of the magnitude of the GCN4-p/AP-1 difference spectrum (Fig. 2*A*). Interestingly, the small perturbation induced by the nonspecific DNA site is consistent with partial stabilization of α-helix.

The DNA-dependent folding of GCN4-p was further examined using a consensus ATF/CREB binding site[15], which differs from the AP-1 site only in the addition of a central CG base pair. In B-form DNA, the two half-sites of this longer element are displaced by a translation of about 3.4 Å and a rotation of 34° relative to their position in the AP-1 site. Although the AP-1 and ATF/CREB sites seem to be targets for distinct families of mammalian leucine-zipper proteins[15], GCN4-p binds to both sites with comparable affinity (Fig. 1). Correspondingly, CD studies of the complex of GCN4-p with an oligonucleotide containing the ATF/CREB site show a folding transition similar to that observed on binding of GCN4-p to the AP-1 sequence (Fig. 3*C*). The CD spectra of both the AP-1 and ATF/CREB DNAs in the region of 250–300 nm are consistent with B-form structure (Fig. 3*D*). Similar, small perturbations in each spectrum are observed on binding GCN4-p. We conclude that the alternative half-site spacings are accommodated by flexibility in GCN4-p rather than by major structural rearrangements in the DNA. Binding to target sites of different length is also observed for *lac* repressor[16], where it is attributed to flexible tethering of individual *lac* headpieces to the core tetramer[17–19].

Our observations by CD spectroscopy of an α-helical folding transition in GCN4-p lead to the following conclusions: (1) the complete GCN4 DNA binding domain undergoes the same dimerization-dependent coil-to-helix transition seen with an isolated leucine-zipper peptide; (2) the basic region of GCN4-p undergoes a coil-to-helix transition when it binds to DNA; (3) α-helical conformation can also be induced in the GCN4-p basic region by lowering the temperature; (4) similar conformational changes are induced in the protein on binding to sites with AP-1 or ATF/CREB consensus elements, implying flexibility in the link between the basic region and the leucine-zipper.

Can our conclusions concerning the coil-to-helix change we have observed when GCN4-p binds DNA be extended to the intact GCN4 protein? We argue strongly that the basic region of intact GCN4 also undergoes such a DNA-dependent folding transition, on the basis of the following observations: (1) GCN4-p binds specifically, with an affinity comparable to that of intact GCN4. Were the basic region of the intact protein 'prefolded,' for example by interaction with segments not present in GCN4-p, its DNA affinity should be higher, corresponding to the free energy expended in folding the peptide; (2) a 60-residue species essentially equivalent to GCN4-p, produced in an *in vitro* translation experiment, binds as a homodimer to a HIS3 target site with the same affinity as a variety of a larger species[4]; (3) in experiments where this 60-residue segment is present with longer chains, large homodimers, heterodimers, and small (60mer) homodimers all bind with the same affinity[5]; (4) our picture of coupled folding and binding is fully consistent with the implications of chemical modification experiments, which show GCN4 and related proteins make extensive contacts with their cognate binding sites[20–22].

These results imply that some part of the DNA-binding domain must be loosely structured to 'wrap' around the DNA helix. One precedent for flexibility in a DNA-binding domain is the N-terminal arm of the λ repressor, which is disordered in the free protein[23]. The arm binds to the 'back side' of the

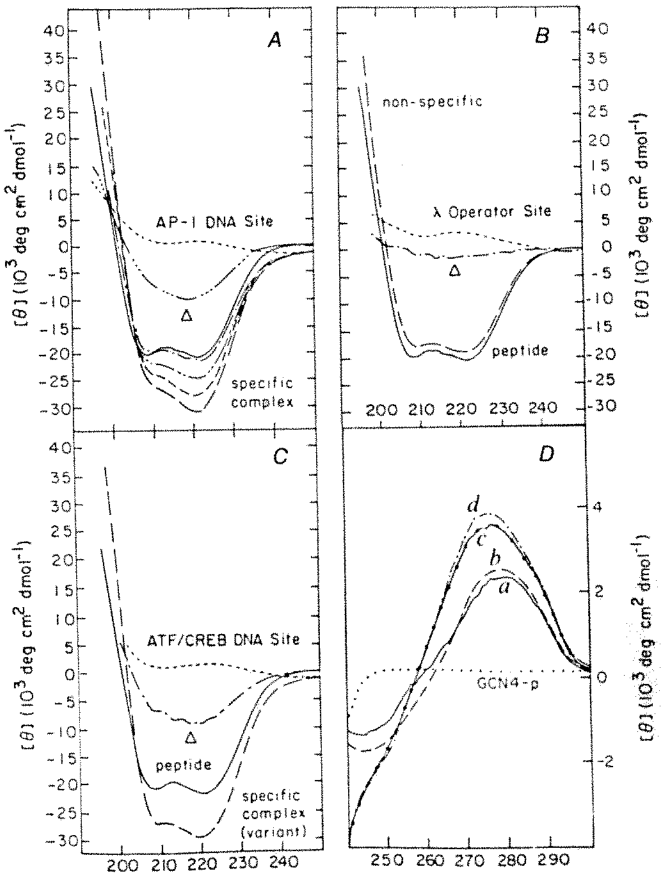

FIG. 3 *A*, Formation of the specific complex with a synthetic AP-1 site results in increased α-helix formation. The GCN4-p concentration was 34 μM. Initial buffer conditions were 50 mM KCl and 12.5 mM potassium phosphate (pH 7.0) at 25 °C. —, the spectrum of GCN4-p is shown; - - -, spectrum of the AP-1 site; — —, spectrum of a 2:1 molar mixture of GCN4-p (one dimer per oligonucleotide duplex) and the AP-1 site (17 μM); — · · · — · · ·, the calculated difference spectrum. Δ is calculated as the difference between the observed spectrum of the complex and the sum of the spectra of its constituents. The lineshape and magnitude of Δ is in accord with a coil-to-helix transition[30]. Dissociation of the specific complex occurs at higher concentrations of KCl: spectra are shown at 500 mM KCl (— —), 700 mM KCl (—-—) and 1,500 mM KCl (—-—). The 21-base-pair AP-1 site has sequence GAGATGAGTCATCTC; a similar DNA-dependent transition is observed with a consensus 15-base-pair AP-1 site. *B*, An unrelated control DNA site (the λ operator site O_L1) does not appreciably affect the helix content of GCN4-p. —, the spectrum of GCN4-p; - - -, the spectrum of the λ operator; — —, the spectrum of a 2:1 molar mixture of GCN4-p and the λ site; — · — ·, the calculated difference spectrum (defined in *A*). The sequence of the λ operator site is GATACCACTGGCGGTGATATC and its complement. *C*, The ATF/CREB DNA site, which contains an additional GC base pair in the centre of the recognition site, induces a similar increase in helix content on formation of a specific complex. —, the spectrum of GCN4-p; - - -, the spectrum of the ATF/CREB site (GAGATGACGTCATCTC) additional central base-pair underlined; the — —, the spectrum of a 2:1 molar mixture of GCN4-p (one dimer per oligonucleotide duplex) and the ATF/CREB site — · · · — · · ·, the calculated difference spectrum (Δ). *D*, Conformation of the AP-1 (spectrum *a*; 15-base-pair sequence described in *A*) and ATF/CREB (spectrum *b*; related 16-base-pair sequence shown in *C*) oligonucleotides may be observed in the near ultraviolet region of the CD spectrum. The ellipticity of GCN4-p in this region (dotted line) is near zero. The spectrum of the AP-1 complex (*c*) and ATF/CREB complex (*d*) show similar small changes in amplitude and position of the local maximum.

DNA helix and forms contacts that, in part, determine the operator specificity of the repressor[23]. In GCN4, the entire recognition region seems to undergo a folding transition on binding DNA. This can be a rather general mechanism for reducing the kinetic barriers in the assembly of protein-nucleic acid complexes.

Our results do not specify the details of α-helical packing in the basic region of GCN4. In the scissors grip model proposed for this class of DNA-binding proteins[20], the basic region forms an α-helical extension of the leucine-zipper, with a kink near the N-terminus. Although consistent with such a model structure, our data are also consistent with an array of α-helical segments within the basic region. Our data do, however, show that the basic region of GCN4 is almost completely α-helical when bound to DNA and that any kinks or turns in the peptide backbone involve very few residues. ☐

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Phylogenetic and genetic evidence for base-triples in the catalytic domain of group I introns

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UNDERSTANDING the mechanisms by which ribozymes catalyse chemical reactions requires a detailed knowledge of their structure. The secondary structure of the group I introns has been confirmed by comparison of over 70 published sequences[1–4], by chemical protection studies[5], and by genetic experiments involving compensatory mutations[2,6,7]. Phylogenetic data can also be used to identify tertiary interactions in RNA molecules. This was first done by Levitt[8], who predicted tertiary interactions in transfer RNA, which were subsequently confirmed by X-ray crystallography[9]. More recently, sequence comparison data have been used to predict tertiary interactions in ribosomal RNA[10]. We have searched a complete alignment of the core regions of group I introns[1,2] for evolutionary covariations that could not be ascribed to classical Watson–Crick or wobble base pairings. Here we describe two examples of phylogenetic covariation that are most simply explained by postulating hydrogen-bonded base-triples similar to those found in tRNA. Genetic experiments with the *Tetrahymena* and *sunY* introns confirm the importance of these interactions for the structure of the ribozyme.

Comparison of group I intron sequences reveals a striking covariation of the second and third base pairs of stem P4 and the first and second nucleotides, respectively, of the single-stranded segment J6/7 (see Fig. 1*a*). Base pair two of stem P4 is most often G·C or C·G; when P4-2 is G·C, the first base in J6/7 is always U; when P4-2 is C·G, J6/7-1 is usually G, less often C, but never U. Several independent examples of concerted changes at these positions are seen in phylogeny (Fig. 1*b*), indicating a functional requirement as opposed to mere evolutionary drift. For example, G·C–U changes to C·G– within both distinct subclasses of the group I introns, showing that this triple mutation has occurred at least twice during their divergence. Similarly, P4 base pair 3 is usually C·G or G·C, and these base pairs are associated with C or U, respectively, at position J6/7-2; again, independent concerted changes of these positions are seen in phylogeny.

Comparing closely related introns can aid in distinguishing between sequence changes that are due to functional necessity as opposed to evolutionary distance. Two bacteriophage T4 introns, T4 *td* and T4 *sunY*, are virtually identical within the conserved core of group I introns[11], yet differ at the second and third base pairs of P4 and the second nucleotide of J6/7. It is unlikely that these sequences would have covaried if the base substitutions were merely the product of neutral drift.

To assess the chemical feasibility of the interactions detected by covariation, we have examined physical models of the proposed triples. All of the phylogenetically related base triples can be modelled as geometrically equivalent, or near-equivalent, structures (Fig. 2). In most cases, hydrogen bond donors and acceptors are interchanged without affecting the geometry of the interaction. The base-triples we propose between P4 and J6/7 are similar to those seen in tRNA[8,9], in that a single base interacts with one base of a Watson–Crick base pair on the major groove face of the base pair, with all three bases being roughly coplanar.

The small number of base changes between the bacteriophage T4 introns *td* and *sunY* made these introns ideal for initial tests of our model. If P4 and J6/7 do interact in a functionally significant manner, changes in one or the other of these regions should be deleterious, whereas changing both together should restore the original structure and enzymatic activity. We started with *sunY* and constructed all possible intermediates on the way to a *td*-like ribozyme, varying J6/7 and the base pairs of P4. The splicing activity of these intermediate forms was measured in the presence of saturating guanosine and the initial velocity of the reaction was determined (Fig. 3). Both intermediates that replace one base triple from *sunY* with the *td*-like base triple are slightly more active than the *sunY* wild type. In contrast, all mutants in which a predicted base-triple is disrupted have decreased activity. Loss of a hydrogen bond from a predicted base-triple (for example, C·G-C to C·G-U) reduces activity

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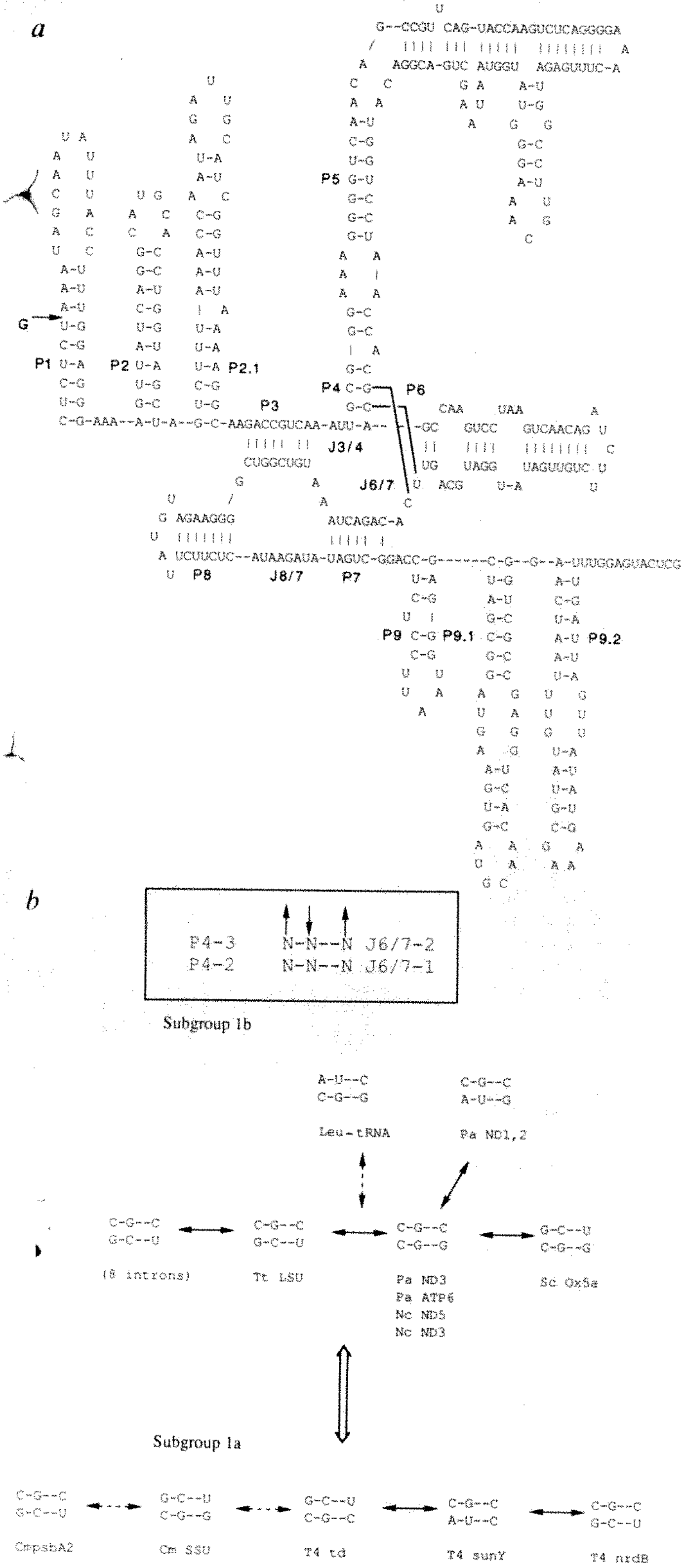

FIG. 1 *a*, The sequence of the catalytic domain of the *Tetrahymena* ribozyme displayed in the universally conserved secondary structure of the group I introns. Paired stems Pn and joining segments Jn/n are labelled according to ref. 13. The proposed base-triples are indicated by bold lines. *b*, Phylogenetic evidence for tertiary interactions within the catalytic domain of the group I introns. The P4 and J6/7 components are drawn to illustrate the proposed base-triples. Introns are arranged in an unrooted phylogenetic tree (F. M., manuscript in preparation), with full arrows indicating a close relationship and dashed arrows a more distant and hypothetical one. Independent instances of triple mutations that change all bases of each of the proposed base-triples can be seen. Names of introns and sequence data are from ref. 1.

by 2- to 5-fold, whereas introducing a steric clash (for example, C·G-C to G·C-C) reduces activity by 9- to 17-fold.

These results also suggest that enzymatic activity is dependent on cooperative interactions between the two levels of triples. The constructs in which only one of the two *sunY* base triples becomes *td*-like, splice 1.3 and 2.8 times faster than *sunY* itself, respectively, whereas the fully *td*-like construct, in which both base triples have been altered, splices almost 14 times faster than the wild-type *sunY* control.

To test further the validity of our structural model, we constructed a variety of base substitutions in a truncated variant of the *Tetrahymena* ribozyme, pAG100, which is particularly sensitive to structural perturbations[12]. As part of a previous study of phylogenetically conserved positions in the catalytic core, we had used cassette mutagenesis to generate all possible single mutations in P4 and J6/7, and all possible double mutations in P4 (ref. 2). Single mutations that disrupt base pairs in P4 are extremely deleterious. Compensatory mutations designed to restore Watson-Crick base pairing sometimes partially restore activity, but are much less active than the wild type. These experiments show that base pairing in P4 is necessary but not sufficient for full enzymatic activity, and support the idea that these base pairs are involved in important tertiary interactions (similar results have recently been obtained by Flor *et al.*, ref. 7). The few allowed alternate base pairs in P4 reproduce configurations already found in phylogeny: for example, changing P4-3 from C·G to A·U, and thus changing the base-triple from C·G-C to A·U-C, results in a configuration seen in the plant chloroplast tRNA leucine introns. Structurally, this substitution switches the hydrogen bond acceptor for the C4 amine of cytosine from the O6 of guanosine to the O4 of uracil.

We used our library of single and double substitution mutations to construct multiple substitutions involving P4 and J6/7. Triple substitutions that would seem to be chemically feasible are generally no more active than their double- and single-substitution parents. To determine why most triple substitutions have only a fraction of wild-type activity, we examined the possibility that only certain pairs of base-triples are compatible, as suggested by our experiments with the *sunY* intron. We constructed a variety of multiple substitutions that simultaneously changed both P4 base-triples (Table 1) by combining double and quadruple mutations in the P4 stem with single or double substitutions in J6/7. In contrast to substitutions that affected only one base-triple, striking suppression of parental phenotypes was seen when both base-triples were altered. The hextuple mutant G·C-U/C·G-G (*ScOx5α*-like) is particularly impressive, as substitutions that had little or no activity in isolation were restored to nearly wild-type levels when combined. No combination of bases on the way to this set is as active as the hextuple mutant itself. Several other suppressions where the activity of a hextuple substitution was greater than either triple substitution were also found.

TABLE 1 Activities of substitutions in P4-2·J6/7-1 and P4-3·J6/7-2

Upper level		Lower level P4-2-J6/7-1		
P4-3-J6/7-2	G·C-U	C·G-U	C·G-C	C·G-G
C·G-C	100 (Tth)	1.2	2.3	1.7
A·U-C	5.6	8.5	16	18 (Leu-tRNA)
G·C-U	0.5	3.6	18 (td)	90 (ScOx5α)
G·C-G	0.1	6.0	—	—

Mutants were constructed in a variant of the Tt LSU intron, pAG100 (described in ref. 12). Assay conditions are as in ref. 2, except that 5 mM spermidine was included to enhance the efficiency of the reaction. Values are expressed as a per cent of the wild type activity. Phylogenetic variants corresponding to the substitutions introduced at these positions are listed in parentheses. A dash indicates value not determined.

The results from *Tetrahymena* and *sunY* lead to several conclusions. First, the structural integrity of a given base-triple is important for catalytic activity. Each of the genetically equivalent triples can be modelled as a chemically feasible structure with rough geometric equivalence (Fig. 2). Combinations of bases that are predicted to lack hydrogen bonds or contain steric clashes are less active than those containing at least one hydrogen bond. Second, it is even more apparent in the *Tetrahymena* intron than in *sunY* that there are cooperative interactions between the base-triples. In all the examples of strong genetic suppression, both triples were coordinately changed. If the effects of mutants in different triples were independent, these recombinant ribozymes should have had even less activity than their parents. Finally, we note that the various successful combinations almost all have corresponding representations in phylogeny (compare with Fig. 1*b*), whereas the less active combinations are not found in nature.

To determine whether the base-triples we have identified are important for all the steps in the splicing cascade, we also constructed a series of mutations in pSZ241 (ref. 12), which contains the wild-type *Tetrahymena* intron flanked by wild-type exon sequences. The substitutions introduced in pSZ241 were identical to several of the hextuple mutants constructed in pAG100 on the basis of evolutionary guidance (Fig. 1*b*). Several of the quintuple or hextuple substitutions (for example, the *td*-like and *ScOx5α*-like combinations) are more active than the quadruple, double and single mutations that went into their construction (data not shown).

The genetic evidence for strong interactions between adjacent base-triples is most simply interpreted as being due to stacking interactions between parallel, stacked base-triples. Such stacked base-triples can readily be modelled as two consecutive elements of a triple helical stack consisting of a classical double-stranded helix and a third strand with approximate A-form geometry interacting in its major groove.

The tertiary interactions that we propose are supported by three lines of evidence. Inspection of a phylogenetic database revealed covariation between the identities of certain base pairs and bases that have fixed locations with respect to the invariant secondary structure. The quality of phylogenetic data is subject to degradation through the inclusion of catalytically inactive introns, and because of sequencing and alignment errors; in addition, not all tertiary interactions need be universally conserved. In view of these potential problems, the correlations that we have observed are particularly striking. Second, we found that these correlated sets of nucleotides could be represented as evolutionary transitions between roughly isosteric base-triples. It is important to note that the phylogenetic data is purely a statistical correlation, independent of any structural consideration, so that the finding that these data fit a consistent structural interpretation constitutes independent evidence in favour of the proposed interaction. Finally, we have used extensive genetic suppression analysis to confirm the importance of these interactions in two quite different group I introns, the group Ia intron *sunY* and the group Ib intron from *Tetrahymena*. Although we believe that these interactions are most simply explained by the formation of base-triples, it is not possible to exclude indirect interactions mediated by water molecules, metal ions or intervening nucleotides. ☐

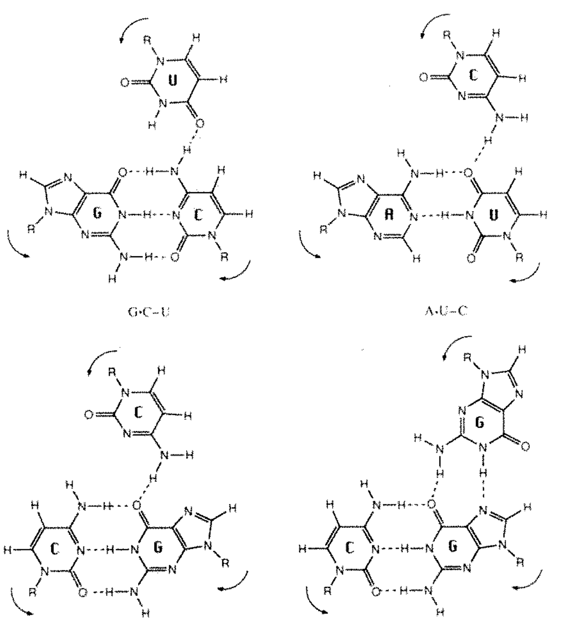

FIG. 2 Models of postulated base-triples discussed in text. Arrows indicate the polarity of the phosphodiester backbone.

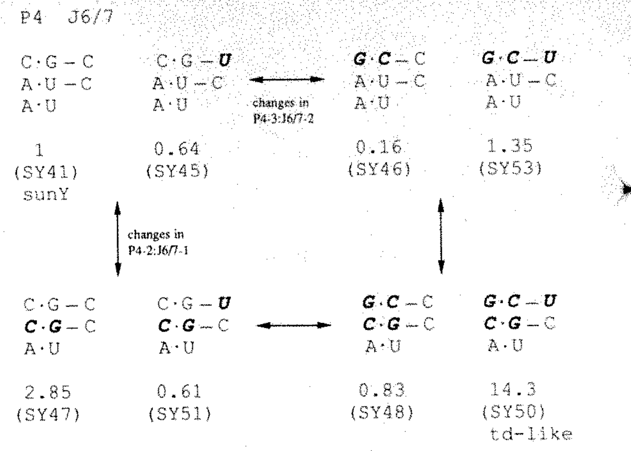

FIG. 3 Mutations in the P4 and J6/7 components of intron *sunY* and their effects on rates of 5′ cleavage. The P4 stem (left) and first two nucleotides of the J6/7 segment (right) are shown for RNAs SY41 (*sunY* wild type) to SY50 (identical to the *td* intron over P4 and J6/7).

METHODS. Mutations were constructed[14] in plasmid SY41 (identical to mutant 4 in Table 1 of ref. 15), which contains the entire core of the *sunY* intron (structural elements P1–P9), and flanking exon sequences, cloned downstream of a T7 RNA polymerase promoter. T7 transcripts of *DraI*-digested plasmid DNA were purified on 4% acrylamide–8 M urea gels. Transcripts were preincubated for 20 min at 45 °C in 50 mM Tris-HCl pH 7.5 (at 20 °C), 50 mM NH_4Cl, 7.5 mM $MgCl_2$, 0.02% SDS. Reactions were started by addition of GTP to a final concentration of 2 mM. Samples were analysed on 5% acrylamide–8 M urea gels. The radioactivity in electrophoretic bands was measured directly with a Betagen scanner (Waltham, Massachusetts) and converted to molar amounts by correcting for the number of G residues. Numbers are initial rates of cleavage at the 5′ intron–exon junction, normalized to wild type, calculated from the time needed to convert 25% of precursor molecules to products (~20 min for wild-type transcripts; all reactions were followed for 90 min, and 25% reaction times estimated either by interpolation or extrapolation, assuming linear kinetics between 0 and 25% reaction). Observed differences in initial rates were exaggerated at lower Mg^{2+} (6 mM), and suppressed at higher Mg^{2+} concentration (30 mM). All mutants yielded the same pattern of splicing products.

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New in 1991

Biodiversity and Conservation

Editors: Alan T Bull, International Institute of Biotechnology, Canterbury, UK; Harold G. Cogger, Australian Museum, Sydney, Australia; Ghillean T. Prance, Royal Botanic Gardens, Kew, UK; Daniel Simberloff, Florida State University, USA.

Biodiversity and Conservation is a new international quarterly journal devoted to the publication of papers on biological diversity in all its aspects, its description, analysis and conservation, and its controlled rational use by man. It publishes research papers on biodiversity and conservation, and incisive articles dealing with the practicalities of conservation management, economic, social and political issues and case studies. The Journal also provides a forum for examining the conflicts between sustainable development and human dependence on biodiversity, especially in such fields as agriculture, environmental management and biotechnology.

Pertinent topics include but are not restricted to: Assessment and monitoring of biodiversity; Captive breeding and relocations; Marine biota; Environmental planning and management; Social and economic constraints on conservation; Genetic diversity; Restoration ecology; New biotechnological product research and development; Responses to environmental change.

Biodiversity and Conservation is multidisciplinary and embraces all life-forms.

ISSN: 0960-3115, Vol 1 (1991), 2 issues.
EC: £46, USA/Canada: $80, R.O.W.: £50.
Individual rates, EC: £19, USA/Canada: $34, R.O.W.: £19.

New in 1991

Pharmacogenetics

Editors: Jeffrey Idle, University of Newcastle-upon-Tyne, UK; Frank Gonzalez, National Cancer Institute, Bethesda, USA.

Pharmacogenetics is a bimonthly international journal starting in 1991 devoted to the rapid publication of research papers, brief review articles, and short communications on genetic variation in response to drugs and other chemicals in humans and animals. At present, papers concerned with these topics are distributed throughout a wide range of biochemical, genetic, pharmacological, toxicological and clinical journals. This Journal will improve communication among the diverse groups working in this area by providing a forum for this research.

An international Editorial Board and strict refereeing ensure that only papers of a high scientific standard are accepted.

ISSN: 0960-314X, Vol 1 (1991), 3 issues.
EC: £60, USA/Canada: $98, R.O.W.: £65.
Individual rates, EC: £25, USA/Canada: $45, R.O.W.: £25.

New in 1991

Applied Financial Economics

Editor: Maurice Peston, Queen Mary and Westfield College, London, UK.

Applied Financial Economics is a new quarterly companion to **Applied Economics**. It will publish research papers, short articles, notes and comments on financial economics, banking and monetary economics with a bias towards practical aspects and applied topics. Particular attention will be paid to the actual operations of financial institutions and markets.

ISSN: 0960-3107, Vol 1 (1991).
EC: £85, USA/Canada: $155, R.O.W.: £95.

New in 1991

Reviews in Fish Biology and Fisheries

Editor: Tony Pitcher, Renewable Resources Assessment Group, London, UK.

Reviews in Fish Biology and Fisheries is the only international journal dedicated to up-to-date reviews of the latest research in all areas of fish and fisheries biology.

Reviews are accepted in any field of fish biology where the emphasis is placed on adaptation, function or exploitation in the whole organism.

The subject matter includes physiology, evolutionary biology, taxonomy, zoogeography, behaviour, ecology, and exploitation. The aims of published reviews are to provide an up-to-date synopsis for research workers in the chosen field with an outline of the next problems which should be tackled. Reviews also provide the non-specialist fish biologist with an awareness and understanding of the field.

In addition to review articles, details of interesting papers given at recent conferences, book reviews and notices of forthcoming conferences are included.

ISSN: 0960-3166, Vol 1 (1991), 4 issues.
EC: £90, USA/Canada: $165, R.O.W.: £100.
Individual rates, EC: £30, USA/Canada: $50, R.O.W.: £30.

New to Chapman & Hall

Glycoconjugate Journal

Editors: Alan Chester, Lund, Sweden; Kenneth Lloyd, Memorial Sloan-Kettering Cancer Center, New York, USA; Toshiaki Osawa, Tokyo, Japan.

Chapman & Hall are proud to announce that **Glycoconjugate Journal** will be published under their imprint from 1991. This well-established Journal will now be published bimonthly, providing rapid publication for important papers at the forefront of research in glycoconjugates and glycobiology. The emphasis is on the metabolism and function of glycolipids, glycoproteins, oligo- and polysaccharides, and proteoglycans. This includes the mechanisms of biosynthesis and biodegradation of glycoconjugates, glycoconjugates in health and disease and glycoconjugate interactions with other molecules.

A new international Editorial Board will enforce strict refereeing standards.

Glycoconjugate Journal is the official journal of the International Glycoconjugate Organization.

ISSN: 0282-0080, Vol 8 (1991), 6 issues.
EC: £130, USA/Canada: $230, R.O.W.: £140.
Individual rates, EC: £90, USA/Canada: $160, R.O.W.: £90.

New in 1991

Processing of Advanced Materials

Editors: Joseph McGeough, University of Edinburgh, UK; Madhav Datta, IBM Corporation, Yorktown Heights, USA.

Processing of Advanced Materials is a new refereed journal publishing original papers of high quality on theoretical, experimental and industrial research and development work in this rapidly growing field of advanced technology.

The Journal will cover all aspects of processing including recently developed methods of forming, joining, machining and surface modification, especially when these techniques are applied to the treatment of advanced materials, such as composites, ceramics, plastics, electronic materials as well as new alloys. **Processing of Advanced Materials** will deal with the fundamentals of processing methods, analytical and numerical models, development of experimental techniques and new industrial practices employed in the manufacturing of advanced materials into usable products.

ISSN: 0960-3158, Vol 1 (1991), 4 issues.
EC: £80, USA/Canada: $145, R.O.W.: £88.
Individual rates, EC: £35, USA/Canada: $60, R.O.W.: £35.

New in 1991

International Journal of Environmental Health Research

Editors: Michael Jackson and Paul Smith, University of Strathclyde, UK; Joan Rose, University of South Florida, USA; Ron Pickett, The Curtin University of Technology, Perth, Australia; Ahmad H. Gaber, University of Cairo, Egypt.

The **International Journal of Environmental Health Research** is an international quarterly devoted to the rapid publication of research in environmental health. The **International Journal of Environmental Health Research** acts as a link between the diverse research communities and practitioners in environmental health. In addition to original research papers, technical notes and review articles, both invited and submitted, are included.

The **International Journal of Environmental Health Research** publishes articles on all aspects of the interaction of the environment with human health.

ISSN: 0960-3123, Vol 1 (1991), 4 issues.
EC: £75, USA/Canada: $135, R.O.W.: £82.
Individual rates, EC: £30, USA/Canada: $54, R.O.W.: £30.

New in 1991

Statistics and Computing

Editor: David J. Hand, The Open University, Milton Keynes, UK.

Statistics and Computing is a new refereed quarterly publishing papers covering the entire range of the interaction between statistics and computing.

Subject areas will include: techniques for evaluating analytically intractable problems such as high-dimensional multivariate integrals; search and optimisation methods; computer-intensive (resampling) methods; simulation and Monte Carlo; graphics; computer environments; reliability of hardware; statistical approaches to software errors; information retrieval; statistics of databases and database technology; statistics and probability in expert systems; image processing; tomography; genetic algorithms; neural networks; reviews of programs, stastistical languages, and packages; macros and algorithms.

Statistics and Computing will also publish book review and software review sections. High standards will be ensured by an international Editorial Board including: D. J. Spiegelhalter, D. Prebigon, J. A. Nelder and B. W. Silverman.

ISSN: 0960-3174, Vol 1 (1991), 4 issues.
EC: £85, USA/Canada: $155, R.O.W.: £95.
Individual rates, EC: £39, USA/Canada: $70, R.O.W.: £39.

New in 1991

Journal of Electronics Manufacturing

Editor: David Williams, Loughborough University of Technology, UK.

Journal of Electronics Manufacturing publishes quality technical papers associated with the manufacturing and assembly of circuit boards, semiconductors and other components and the integration of these into products. Related subjects in materials and design are covered and important issues in business and management within the electronics industry are addressed. **JEM** is an independent, vertically integrated journal aimed at providing a unique interface between managers, researchers and application engineers working both in the academic and industrial communities.

JEM will publish invited keynote papers, original research papers, state-of-the-art reviews, tutorial reviews, industrial case studies, conference reports and book reviews.

ISSN: 0960-3131, Vol 1 (1991), 2 issues.
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Individual rates, EC: £19, USA/Canada: $34, R.O.W.: £19.

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Monthly from 1991

Journal of Neurocytology

Editors: Bob Lieberman, University College London, UK; Milton Brightman, National Institutes of Health, Bethesda, USA.

Now published monthly the **Journal of Neurocytology** publishes papers concerned with the structure and function of nervous tissue. The scope of the Journal embraces the organization and fine structure of neurons, receptors and glial cells, synapses and other intercellular specializations, including associated biochemistry, cytochemistry, pharmacology, physiology and biophysics, and extends to both vertebrates and invertebrates under normal, experimental and pathological conditions. Papers reporting multidisciplinary studies are particularly welcome.

ISSN: 0300-4864, Vol 20 (1991), 12 issues.
EC: £345, USA/Canada: $595, R.O.W.: £380.

Twice Monthly from 1991

Journal of Materials Science and Journal of Materials Science Letters

Editor: William Bonfield, Queen Mary and Westfield College, London, UK.

These two companion journals are now firmly established as the leading sources of primary communication for scientists investigating the structure and properties of all engineering materials. **JMS** will publish 30% more reviews and full-length papers in 1991, in 24 issues. **JMS Letters** will also publish 24 issues, using fast-track production schedules to ensure rapid publication.

In 1991 subscribers will receive more than 9000 pages of top-quality research in 24 issues each of **JMS** and **JMS Letters** and 4 issues each of **JMS: Materials in Electronics** and **JMS: Materials in Medicine.**

ISSN: 0022-2461, Vol 26 (1991), 56 issues.
EC: £1350, USA/Canada: $2395, R.O.W.: £1450.

New to Chapman & Hall

Ada User

Editor: Dan Simpson, Brighton Polytechnic, UK.

Ada is the single most important development in computer programming of the last decade and the NATO standard for real-time system projects. Ada User publishes papers, short articles, working notes, conference reports, news items, correspondence and book reviews on the development and use of Ada. Ada User is the official publication of Ada UK but its content is of vital interest to all those who work in Ada worldwide.

ISSN: 0268-652X, Vol 12 (1991), 4 issues.
EC: £70, USA/Canada: $125, R.O.W.: £77.

New Title in 1991

Journal of Property Research

Editors: Bryan MacGregor, University of Aberdeen, UK; David Hartzell and Mike Miles, University of North Carolina, USA.

The **Journal of Property Research** is the only international journal covering all aspects of real estate investment and development.

Formerly **Land Development Studies**, the Journal's title has been changed to reflect the enormous expansion of research, particularly applied research, into property investment and development.

The **Journal of Property Research** publishes theoretical and empirical papers, case studies and critical literature surveys, regular editorials, book reviews and market briefings. The focus may be financial, economic or environmental, urban or rural and public or private sector.

ISSN: 0264-0821, Vol 8 (1991), 3 issues.
EC £98, USA/Canada: $175, R.O.W.: £105.

17% More in 1991

Evolutionary Ecology

Editor: Michael Rosenzweig, University of Arizona, USA.

Evolutionary Ecology is a journal of basic concepts at the interface of evolution and ecology. It publishes theoretical and empirical papers of original research. It includes genetical, behavioural, palaeobiological, physiological, population-level and community studies, regardless of the taxon or biome from which they are drawn. It will publish 17% more material in 1991.

ISSN: 0269-7653, Vol 5 (1991), 4 issues.
EC £110, USA/Canada: $198, R.O.W.: £120.
Individual rates, EC: £35, USA/Canada: $58, R.O.W.: £35.

Bimonthly from 1991

Journal of Intelligent Manufacturing

Editor: Andrew Kusiak, University of Iowa, USA.

Now bimonthly, the **Journal of Intelligent Manufacturing** is a new refereed journal publishing quality papers on applications of artificial intelligence in manufacturing. It informs readers on the effective design, development and use of intelligent systems.

The Journal features new methodologies, developments, case studies, surveys and tutorials in manufacturing and acts as a vital link between the research community and practitioners in industry.

ISSN: 0956-5515, Vol 2 (1991), 6 issues.
EC: £95, USA/Canada: $170, R.O.W.: £105.
Individual rates, EC: £45, USA/Canada: $80, R.O.W.: £45

New Title in 1991

Geotechnical and Geological Engineering

Editor: David Toll, University of Durham, UK.

Previously published as the **International Journal of Mining and Geological Engineering**, this Journal is being relaunched to reflect the increasing amount of top-quality research being done in geotechnics and geological engineering. Its emphasis is on practical applications with priority given to: case histories describing ground engineering projects, including contractual and logistical aspects; novel geotechnical construction techniques; pollution and environmental problems; tropical soil and rock engineering; ground investigation and computer aided geotechnical and geological engineering including the application of information technology.

ISSN: 0269-0136, Vol 9 (1991), 4 issues.
EC: £120, USA/Canada: $215, R.O.W.: £132.

New Journals in 1992

Transgenic Research
Journal of Design and Manufacturing

Monthly from 1991

Journal of Applied Electrochemistry

Editor: Tony Wragg, University of Exeter, UK.

Now monthly, the **Journal of Applied Electrochemistry**, is the leading Journal on all aspects of electrochemistry. The interface between electrochemical science and engineering is highlighted, emphasizing the application of electrochemistry to technological development and practice.

ISSN: 0021-891X, Vol 21 (1991), 12 issues.
EC: £350, USA/Canada: $630, R.O.W.: £385.

Double the Size from 1991

Applied Economics

Editor: Maurice Peston, Queen Mary and Westfield College, London, UK.

Applied Economics is the only monthly economics Journal, it encourages the application of economic analysis to specific problems in both private and public sectors. In 1991 it is increasing in page size and number, doubling the amount of material published.

ISSN: 0003-6846, Vol 23 (1991).
EC: £395, USA/Canada: $730, R.O.W.: £430.
Subscription includes 14 issues of Applied Economics and 4 of Applied Financial Economics.

42% More in 1991

Transition Metal Chemistry

Editor: David Walton, University of Sussex, UK.

This leading Journal on all aspects of the chemistry of transition metals will publish 42% more material in 1991.

ISSN: 0340-4285, Vol 16 (1991), 6 issues.
EC: £295, USA/Canada: $530, R.O.W.: £325.

Bimonthly from 1991

Construction Management and Economics

Editor: John Bennett, University of Reading, UK.

Construction Management and Economics is an international journal serving all professional practitioners and researchers in the construction industry. From 1991 it will be bimonthly.

ISSN: 0144-6193, Vol 9 (1991), 6 issues.
EC: £160, USA/Canada: $285, R.O.W.: £175.

Quarterly from 1991

Journal of Sports Sciences

Editor: Tom Reilly, Liverpool Polytechnic, UK.

Journal of Sports Sciences publishes top quality papers on all aspects of sports science including: biomechanics, sports psychology, kinanthropometry, physiology and sport performance. It will publish 4 issues and 33% more material in 1991.

ISSN: 0264-0414, Vol 9 (1991), 4 issues.
EC: £125, USA/Canada: $225, R.O.W.: £137.

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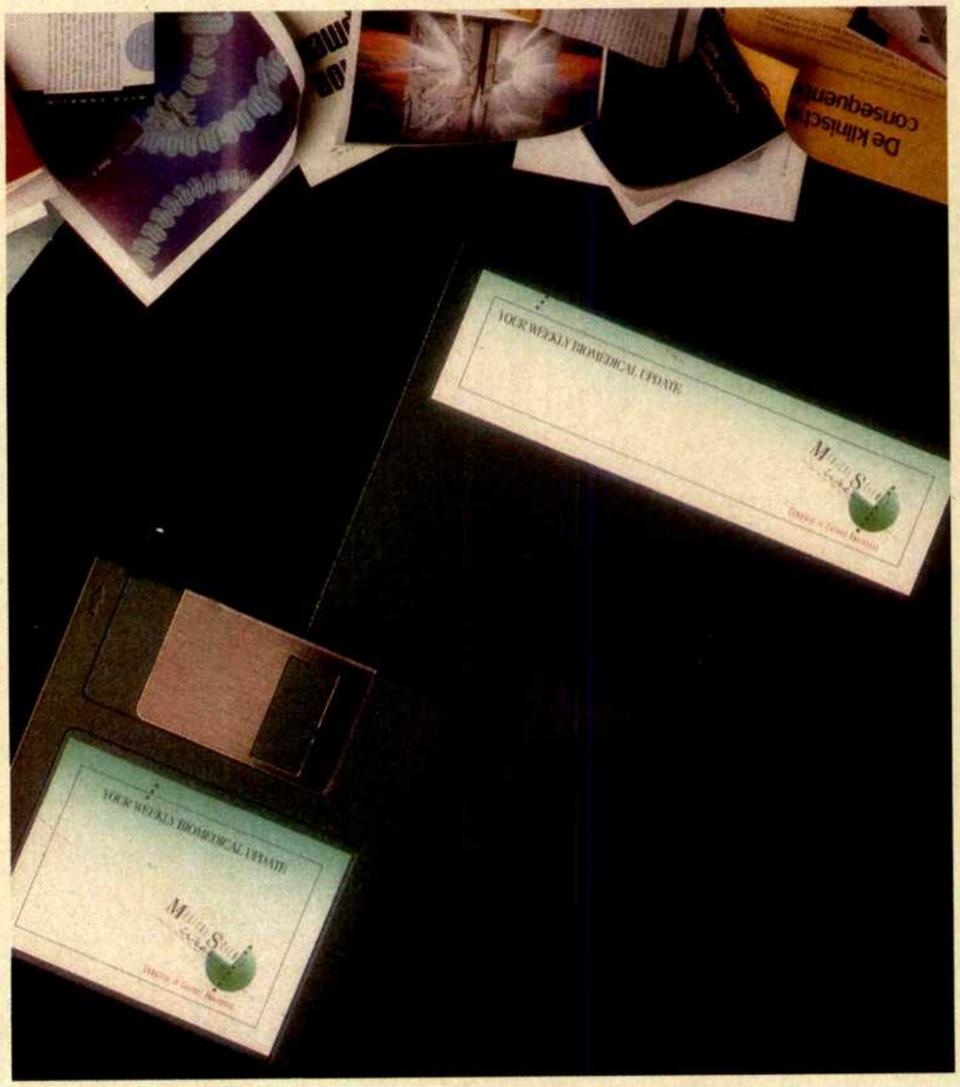

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Communication problems

Michael Buckingham

The gap between the promise of electronic information systems and practice is shrinking. But slowly.

For almost two decades we have been threatened with a revolution in the communication of technical information to be brought about by electronics. So far, however, the casualties have been confined to the revolutionary armies themselves.

One notable example is the American Medical Association, which is pulling out of a scheme to run an on-line information service for its members. With losses reportedly running at $250,000 a month, nobody can accuse the AMA of not trying. The venture did not fail from lack of initial interest; registrations were well up to expectations. Rather, few potential users were prepared to overcome the 'hassle factor' of telecommunications with any regularity. The point is this: if an organization with the money, power and influence of the AMA, and an audience well able to claim tax relief on expenditure, cannot make such a service work, what hope is there for penetration of electronic systems into the beleaguered academic market?

The importance of convenience is highlighted by the interest in professional services reported by the French Minitel operation, which offers a freely distributed base of five million Minitel videotex terminals. Access to the Medline database in the United States through Minitel is now being heavily promoted, with telephone companies in Germany, Italy and The Netherlands planning to join in the distribution of terminals free or at low cost. (By contrast, the value of supporting an information and communications infrastructure has so far been lost on the British government.)

Research results

Developments such as these have had little bearing on the main area of concern for the scientist, the communication of primary research results. The principal arguments for electronic systems — rapid communication and rapid response — still hold good, and are strongest when information can be presented concisely and where the audience has the courage to respond off the cuff. But there remains a mismatch between the volumes of information considered essential in a scientific paper and the volumes which can easily be handled by computer networks and conveniently printed. The merits of short communications must be clear to readers of this journal at least — if not its contributors.

This is a challenge to which technology may be rising faster than expected. The arrival of high-speed digital communications in the form of integrated services digital networks (ISDNs) holds the possibility of transmission of papers in image form at high speed and acceptable cost. One consequence may be that the much-discussed Adonis project (see *Nature* **344**, 287; 1990), which is based on the delivery of papers in image form using the medium of optical disks, will be outdated even before it is fully commercialized.

High-speed digital communications will certainly benefit full-text databases, which so far have grown steadily but not spectacularly. The US database hosts BRS and STN have continued to put large resources behind building collections in medicine and chemistry; BRS, for example, now has over 90 titles in its collection. This number is small in comparison to the 400 titles planned for Adonis which, in turn, is only a fraction of the total biomedical literature. Yet the volume of material that Adonis can carry is still constrained by the physical problems of journal scanning, disk mastering and distribution.

A considerable step forward in providing a broader service would be to integrate the efforts that now go into the collection and processing of journal issues for major abstracting services with the scanning and distribution on demand of papers in image form through ISDNs. This would both increase the volume of titles and free the distribution process from the need for physical access to an Adonis workstation. Although Adonis has now moved to industry-standard hardware, the physical constraints remain substantial.

The main question to be considered is a political one: who would control an integrated service such as this? Capital and system development would be substantial and it is not at all clear that it would be possible to support many competing systems. The real importance of Adonis may be that it has served to unite a collection of publishers into a group able to counter the power of on-line hosts. Whether a hard-pressed library community will be able to combine to represent the consumer remains to be seen.

What of the 'end user', the scientist at the bench? The costs and complexities of access to on-line services have combined to hold back growth in this market, the information professional continuing to be the gatekeeper. Disk-based services have, however, grown rapidly; for example the penetration of Medline CD-ROMs into hospitals and medical research centres is now close to complete, although researchers in the basic sciences are generally less favoured.

Modern methods of data retrieval are undoubtedly more comfortable to use than manually thumbing through printed indexes, but the indexing and retrieval process has not advanced to any comparable degree. The sheer volume of material remains overwhelming. Like motorists we are locked into a situation with better and better cars travelling more and more slowly on more and more crowded roads. Against this background, the growth in various forms of print-on-paper review publications is hardly surprising — the immediate need is not for more information, but for selection, evaluation and analysis.

Social networks

At the heart of the matter is a failure to build effectively on existing foundations. Scientific meetings are said to be where you go to meet people you don't know you need to meet. An electronic service will represent a real advance when it can help you find out what you don't know you need to know. To do so it must combine exchange of formal knowledge with the cut and thrust of debate. In other words, electronic networks need to be built on social networks.

As in so many other fields, the model approach could well be found in Japan. The National Academic Centre for Science Information Systems (NACSIS) in Tokyo provides a private network linking universities, colleges and national research institutes. Besides providing basic bibliographic services and access to conventional databases, NACSIS offers facilities for the exchange of research work by electronic mail, bulletin boards and fax, and in its breadth of coverage and attention to customer needs is now going well beyond the similar types of networks to be found elsewhere. The combination of convenient access to the formal literature and informal communication within a relatively tight-knit community will prove enormously powerful.

But the nature of the communication process in science, which still rests upon peer review and the delivery of the printed word, remains largely unchanged. Considering the interval between the invention of printing and the advent of the first scientific journal, we have all been over-optimistic in welcoming the New Age of information. ☐

Michael Buckingham is Director of Database Publishing at Adis International, 41 Centorian Drive, Mairangi Bay, Auckland, New Zealand.

Choice for the consumer

Peter Sheterline

Current Opinions in Cell Biology. Editors N. B. Gilula and L. Wolpert. *Current Science. 6/yr. North America $185 (institutional), $85 (personal); Japan ¥41,250 (institutional), ¥18,750 (personal); elsewhere £110 (institutional), £50 (personal).*

THERE are two extreme ends to the spectrum of philosophies regarding the publication of scientific data (and of anything else for that matter): the Calvinist view which regards publication of anything less than a 'complete' piece of work as opportunistic; and the liberal view that all (refereed) experimental information has value and that any other position smacks of censorship. For publishers, of course (excluding those mediating the activities of scientific societies), the latter position is implicit in the process of running a publishing business, providing that the publications can be sold; for administrators of libraries, quite the opposite view is convenient in the context of diminishing resources (see Merrimen's article in last year's journals issue, *Nature* **341**, 349–350; 1989). For the scientific community itself, and therefore for the benefits to society that accrue from science, the forces that determine 'quality' and quantity of publication are complex, as in the ecology of science, the number of papers published, the ability to obtain funds to do the research leading to publication and the career opportunities for individual scientists are all interlinked. Paradoxically, the suppliers of information in this publishing system are also the consumers and the objectives in each role may be different. Thus, although like all ecologies it is intrinsically self-adjusting and basically conservative, the increasing pressure for individuals to publish may have driven the system to one that benefits the supplier but not necessarily the consumer.

The size of the professional scientific community, and hence of its output of data, has grown out of all recognition from the community of gentleman (mostly) scientists of the nineteenth century. This, and the increasing pressure on scientists to publish regularly to avoid perishing, has led to the large increase in the number of scientific journals (as evidenced by this annual section in *Nature*) and a steady shift away (in terms of numbers) from general to audience-specific journals. As has often been noted, this results in real difficulties for current awareness of scientific information and tends to drive the formation of self-appointed elites that influence the content of the 'high profile' journals and thereby determine fashionable paradigms. The counter force to these events has been the growing availability of computer-based search facilities and the emergence over the past few years of opinion and trends type journals. This, it seems, permits a victory for the liberals.

Cell biology, that amorphous discipline which has clasped morphology, biochemistry, biophysics and latterly molecular genetics to its bosom, illustrates well the evolution of proliferative publishing. The earlier trend towards specific journals on membranes, supramolecular structures(?), calcium and so on, while recognizing the concentration of effort in particular fields, did not easily satisfy the need for cell biologists to maintain some kind of holistic perspective on cell behaviour and function, arguably, the *raison d'etre* for cell biology. Does the recent introduction of *Current Opinion in Cell Biology* and will the projected *Trends in Cell Biology* help redress the balance?

Many cell biologists will have already reached their own conclusions. The first issue of *Current Opinion in Cell Biology* was published at the beginning of 1989 and is modestly priced to encourage personal subscriptions. It was the brainchild of Vitek Trecz, a publishing entrepreneur, and emerged from a similar set of overview journals aimed at the medical community. The journal is released in six issues per annum, each of which contains current overviews of different areas of cell biology; for example, the first is entitled "Cytoplasm and cell motility", the second "Cell multiplication and cell regulation". Each subject area has a section editor who gathers together a group of relevant authors whose remit appears to be to comment on the current status in their discipline in the light of data published in the previous year. The authors are supported by a bibliography of "world literature" in the field from the previous year (in fact in the period of about 4–16 months before publication) gathered by an independent group of literature sleuths. The main bibliography, though large, is not complete, but has been sifted for relevance.

As a consumer scientist and teacher, I find this journal extremely useful. It is a rolling record of overview and comprehensive literature source, which can be conveniently picked off the shelf for entry into the literature base in any of the main areas of cell biology. I have enough shelf space for the next few years' worth. It is particularly useful for final-year undergraduates and for graduate students. I am less impressed by the overviews themselves, mostly because (presumably in deference to the journal's title) they are opinions. Although they are constrained in length (four printed pages) it would be more useful if they were used to establish the key information in the field as of then (perhaps we could call them reviews?) and to leave detailed discussion of particular aspects of the field (mainly of value to a relatively small band of specialists) to the excellent literature source. There would then be a rolling record of the current positions in the key areas of cell biology in

New journals review 1990

THE following explanation is offered to those wishing to know the basis on which the journals mentioned in the following pages were chosen for review.

In *Nature*'s previous journal supplements*, two principles determined the time criteria for a publication to be eligible for review in a particular year. First, that at least four different issues should have been published, to give reviewers a reasonable sample on which to base a judgement. Second, that most new journals start as quarterlies though they often later become more frequent.

Thus in last year's review supplement journals first appearing after May 1987 and with four issues available by May 1989 were considered for review. The first date was carried forward from the previous year; the second meant that four issues of a quarterly would be available to reviewers at the time of commissioning reviews in June 1989.

Other criteria were that the journal should appear at least three times a year, and that the main language used should be English. Abstracts publications and newsletters are not reviewed. Several titles eligible for review are not covered here; a list of them appears on page 599.

The brief given to the reviewers was to limit themselves to comment on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample of issues, and apply to mid-1990 at the latest. As in previous years, the preponderance of journals in the biological sciences reflects the bias of material submitted for review.

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1990. This information is not complete in all cases, and readers interested in subscribing to a particular journal should check the rates with the publisher concerned. ☐

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* See *Nature* **341**, 350–370 (1989); **335**, 459–478 (1988); **329**, 357–376 (1987); **323**, 359–379 (1986); **317**, 293–308 (1985); **311**, 309–330 (1984); **305**, 477–497 (1983); **299**, 491–514 (1982) and **293**, 341–369 (1981).

one place and upon which new developments could be hung. Most areas could expect an airing annually or at least every two years, if the journal remains at its present size. Such review material, although reiteration of basic information to practitioners in the field, requires their wisdom for compilation. Such a potted literature service is, and will increasingly become, a useful companion for cell biologists. ☐

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Laboratory practices

Steve S. Sommer

Technique. A Journal of Methods in Cell and Molecular Biology. Editors Peter Little (UK) and Stephen Hughes (US). *Saunders Scientific. 6/yr. US $210 (institutional), $125 (personal), $85 (student); European Community £157 (institutional), £100 (personal).*

Methods in Molecular and Cellular Biology. Editor in chief Bernard Perbal. *Wiley-Liss. 6/yr. US $125; elsewhere $175.*

HAVE methods become our madness? In the beginning of my scientific life, there was a strong oral tradition, a few established books and journals such as *Methods in Enzymology* and *Analytical Biochemistry*, and methods papers appeared only sporadically in the literature. Curiously, these papers were viewed by some as second-class publications. As the number of individuals engaged in molecular biology grew and the pace of research accelerated, a thirst for additional sources of methods developed. In 1982, Maniatis, Fritsch and Sambrook codified much of the previous oral tradition in their now classic "blue bible". A myriad of non-traditional sources appeared such as the "for the record" section in *Nucleic Acids Research*, and periodically updated protocols were published (such as Wiley's *Protocols in Molecular Biology*). Biotechnology suppliers such as BRL began publishing newsletters focusing on methodology, which were mailed free to anyone wanting a copy. They contained useful articles and they were a hit! Now, virtually all suppliers provide free newsletters, and journals such as *BioTechniques* have emulated this approach.

Has all this activity quenched our thirst for methods? The editors of *Technique* and *Methods in Molecular and Cellular Biology* (*MMCB*) are betting that it has not. *Technique* is a new journal that publishes methodology reviews, subject overviews, protocols (bench-ready protocols of previously described methods), original contributions and short communications. Dates of receipt and acceptance are not provided so that the rapidity of publication cannot be assessed.

As stated in the inaugural issue of *Technique*, the goal of this journal is to publish "detailed descriptions of methods, discussions of sources of failure, sources of error, or ease of application". These goals are generally met. Most laudably, the articles are concise, and the protocols are boxed and written to be "bench friendly". The co-editors have assembled a stellar editorial board including an international array of individuals who have made seminal contributions to the methodology of molecular biology. But this board needs to be used more effectively. Some of the "original contributions" are at best minor modifications of previous methods which should have been published in the "protocols" section with citation of the previous work. New journals such as *Technique* and *MMCB* can establish credibility only by carefully separating the original contributions from rehashes of previous work.

MMCB publishes reviews, original contributions, commentaries on protocols (describing difficulties with or improvements to published protocols, applications of published protocols to other fields or requests for information on obscure points in procedures), and "nuts and bolts" ("letters from readers wishing to comment on the quality, usefulness, and applicability of methods published in the journal and of commercial products in molecular biology"). For commentaries and nuts and bolts, replies from the authors and companies are generally published in the same issue.

There were only a few examples of commentaries and nuts and bolts in the four issues I was sent for review, and these were unimpressive, but it seems premature to pass judgement on their ultimate usefulness. Again, rapidity of publication cannot be assessed because the listed journal dates predate the acceptance dates on the manuscripts. The editorial board is broadly based, with representation from countries such as mainland China and Czechoslovakia. Few articles dealt with areas of my expertise so it was harder for me to judge the effectiveness of editorial review.

Each issue of *Technique* or *MMCB* contains six to nine articles. Both journals can offer sustenance to those who still thirst for methods. Only time will tell how much thirst will remain after partaking of the established brands. ☐

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Bid for the top

Jeremy Hyams and David Latchman

The New Biologist. Editor in chief Arthur S. Levine. *Saunders. 12/yr. US $125 (personal), $85 (student), $225 (institutional); elsewhere $160 (personal), $120 (student), $260 (institutional).*

THE *New Biologist* was launched in October 1989 with the statement that "virtually all of the biological sciences are converging on two central phenomena, namely, signal transduction and differential gene expression" and the promise to "deliver to you significant interdisciplinary papers from a broad readership". Almost one year on, it is interesting to see how far the editors have justified the former and how close they have come to achieving the latter.

The New Biologist publishes three types of papers: research reports, research reviews and meeting reviews. In addition, there are occasional essays under the heading "Theory and hypotheses", and an "Editorial forum" which has varied from reviews of the 1989 biological Nobel prizes to a rather indulgent homily on scientific creativity. It is the balance or, perhaps more accurately, the imbalance, between these various headings that is most striking. Clearly, any new journal lives or dies by the quality and quantity of its original papers. Meeting reviews can be interesting and informative, but they are no substitute for high-quality original research reports. *The New Biologist* has tried to cope with the slow paper flow that any new journal experiences by appointing an editorial board of truly Cecil B. de Milleian proportions. Given this, it is disappointing that the reasonable numbers of research papers in the early issues have declined significantly, with more recent issues containing only four such papers and, hence, dominated by the other sections.

Although the editors of *The New Biologist* must therefore have some qualms about the quantity of its output through its first year, the quality is extremely high. This is matched by the production which lies, perhaps not entirely coincidentally, somewhere between *Cell* and the *EMBO Journal*. Quite where *The New Biologist* sees itself in the cell and molecular biology journals market is in fact quite interesting. On the one hand it has made a serious, and to a significant extent successful, bid for the sort of top-of-the-line research that might hitherto have gone to the journals mentioned above or even graced these hallowed pages. On the other hand, the review articles are occasionally written in a style that owes more to a Murdoch tabloid ("The pinheads and fuzzbrains go haywire") than a serious heavyweight. The editors should be encouraged to keep their heads and maintain the high standards they have set for research articles. If the quality can be maintained the quantity will surely follow. ☐

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Growing pains

M. J. O. Wakelam

Cell Growth & Differentiation. Edited by George F. Vande Woude *et al. American Association for Cancer Research. 12/yr. US $150 (institutional nonmembers), $90 (individual nonmembers).*

Growth Factors. Editor in chief Antony Burgess. *Harwood. 4/yr. North America $190 (corporate), $130 (university), $66 (individual).*

Progress in Growth Factor Research. Executive editor John K. Heath. *Pergamon. 4/yr. DM 285.*

INVESTIGATION into the mechanisms involved in cell growth is one of the most heavily funded areas of modern biomedical research. Thus it is hardly surprising that over the past few years the number of journals covering this area has also appeared to proliferate. The three latest additions, *Cell Growth and Differentiation*, *Growth Factors* and *Progress in Growth Factor Research* hope to fill perceived gaps in the present journal field. To this end each has assembled editorial boards consisting of well-respected, active researchers which, not surprisingly, overlap somewhat. Each journal is also to be praised for not instituting page charges, a serious obstacle to British scientists since the agencies supporting us do not provide publication costs.

One of the impressive aspects about research in the fields of growth-factor structure, function and action is the interactions that have taken place between people working on the different aspects, such as between those studying the molecular biology of growth-factor action and those investigating the signal-transduction pathways. This degree of communication could imply that specialist journals may have a role in increasing such interactions. But the initial impetus in each case was the result of different groups publishing in widely read general journals in the first place, which would tend to argue against such a role for specialist journals.

Cell Growth and Differentiation is described as the molecular biology publication of the American Association of Cancer Research. A large proportion of the papers in the first six issues deals with oncogenes, though the editors hope to encourage papers on other aspects of growth and differentiation research — indeed, the composition of the editorial board should help in this. Some issues contain a "research capsule", which is designed to provide an update on new concepts and methodologies, and which I found both informative and relevant. Nevertheless, it is difficult to see this journal making a major impact: most of the papers it has attracted so far and those it appears to be targeting are generally found in existing specialist journals such as *Oncogene*, *Oncogene Research*, *Molecular Cell Biology* and *Journal of Cell Biology*. There does not really seem to be a need for yet another journal in this area.

Growth Factors hopes to carry papers covering all aspects of research into molecules which control the proliferation and differentiation of cells. It seems to have been successful in this aim so far, having published papers on the genes, synthesis, purification and function of growth factors. Each issue carries a wide cross-section of interest, and a commendable aspect is the profusion of colour illustrations, of particular importance for immunohistochemical studies. But despite the journal's plan to focus on growth factors, one is left with a feeling of a lack of specialization. Most of the recent articles have tended to be concerned with polypeptide growth factors such as fibroblast growth factors, insulin-like growth factors and transforming growth factors. Perhaps this could become the primary focus of *Growth Factors*, otherwise there is a deal of similarity with several cell biology journals, which makes one doubt if there is a niche for this journal to occupy.

In addition to journal proliferation, the multiplication in the number of journals either devoted to, or carrying, reviews has got to the stage where it seems that a large proportion of the research community must be writing, or have recently written, a review. Thus is there a need for *Progress in Growth Factor Research*? Surprisingly, perhaps, I think there is. Despite the efforts of journals such as *Growth Factors* and *Cell Growth and Differentiation*, it is not possible to keep up with the literature covering all aspects of growth-factor research. There is a need for a journal containing up-to-date, short reviews to help today's somewhat narrow researcher to be aware of new developments and concepts in the wider area of growth factors. The first four issues of this journal carry reviews of topics ranging from clinical trials to oncogene structure and function. The reviews are written by active workers in their fields and seem to be published soon after preparation — it is refreshing to read a review which contains references to papers published in the same year. The articles do not contain pages of history but do focus on the key, up-to-date pieces of information, which is what is required of a good review journal. *Progress in Growth Factor Research* appears only quarterly, which should maintain the topicality of its articles. Provided the present standard is maintained, this should be a useful journal for those in the field. ☐

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Antipodean issues

John Galloway

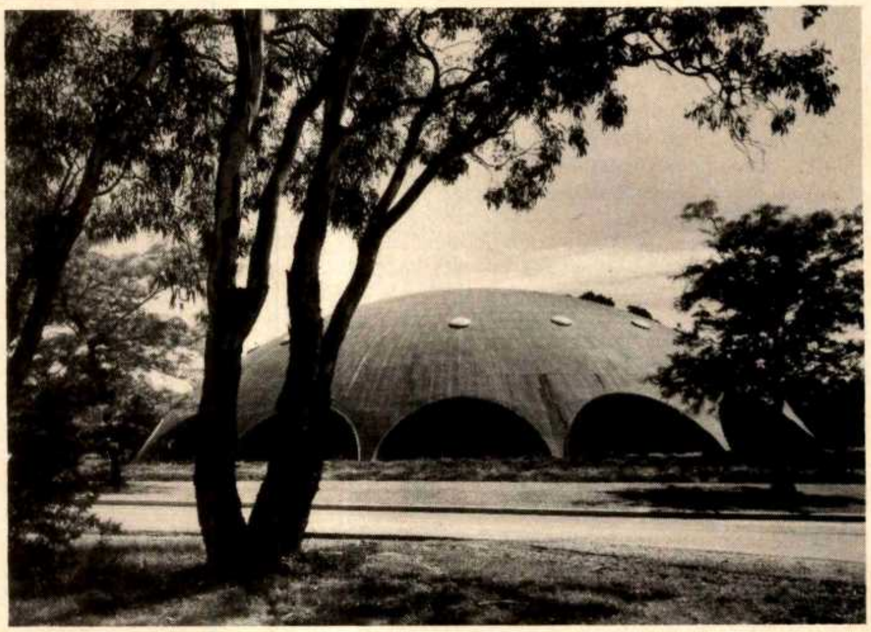
Today's Life Science. Editor Jeremy Knibbs. *Thomson Publications, Australia. 12/yr. Australia A$56, rates elsewhere available on application.*

FOR a moment the title fooled me, but the advertising gave it away. "Life" is not to be understood in the sense of "life on Earth" but rather as in "life and death". This is not *Trends in Biological Sciences,* it is a collection of reviews of molecular medicine for those Australians who make their living at it. The best test for a readership of any journal or magazine is its advertising. *Today's Life Science* carries a lot of high-tech and bio-tech adverts and this I take it defines its audience. Although I couldn't help wondering how big this audience might be, the advertisers clearly think it is big enough.

The format is fairly glossy and the formula is simple — half a dozen reviews in each issue categorized by medical discipline: biochemistry, haematology and microbiology. The articles taken *en masse* reveal how outmoded are medicine's traditional disciplines and how arbitrary their boundaries in the age of molecular biology. A further review covers methods and the final one, "Agenda", addresses a problem that adds some other dimension to the straight science — ethical, educational or economic. Most issues of the journal also carry a feature called "Viewpoint" which as far as I could gather overlaps with "Agenda" in its choice of subjects, but its tone seems more partisan. A number of pages are also given over to "Current Topics" — short pieces mainly on science but leavened with political or social observation.

This is a journal rather than a magazine; the material is largely, perhaps exclusively, written by working scientists rather than by the editorial staff. Inevitably, it has a parochial feel to it. It is written by Australians for Australians, and an emphasis on Australian research is one of the guidelines for authors. Some of these authors are Australian by adoption rather than by accident of birth: I was glad to see an article about heparin by Joan Dawes who not that long ago was helping run the Scottish National Blood Transfusion Services Headquarters laboratory in Edinburgh.

The first issue covered receptor control of haemostasis, tumour necrosis factor, the molecular approach to a cholera vaccine, the pyruvate dehydrogenase locus and automated DNA sequencing. Later issues are similar. The agenda item for the first issue was "biomedical education and research in crisis": poor salaries, a lack of career structure and funding problems. I couldn't think for the moment where I'd heard that before. In fact, a strong sense of *déjà vu* accompanies the reading of many of the articles that have strayed from straight science. A government gift horse well deserving of a long hard look in the mouth, for example; good Australian ideas not reaching the market place; priority elbowing out patents; the poor image of science on television. It is all pretty familiar.



The headquarters building of the Australian Academy of Science in Canberra. The body of the building is contained inside a huge copper-sheathed dome 47.5 metres in diameter, resting on arches set in a moat.

Australian Information Service.

One article that managed to break away to some extent from the utilitarian or from engaging in recrimination about government was a piece about Australian involvement in the human genome project. Not content with pointing out the enormity of the task of sequencing the three thousand million base pairs of human DNA involved, the authors suggest that what we really want to sort out is the genome of the total biosphere, all 10^{14} base pairs, before it is too late. A nice touch was the piece looking forward to a visit to Australia by Jim Watson — it could have been Frank Sinatra coming to perform a concert or open a holiday resort.

I can see — or think I can see — why Australian molecular medicine might need a journal like this. It is really a sort of round robin keeping what is presumably a rather scattered tribe of scientists in some sort of contact with each other. Australia has some excellent molecular medicine and it is worth keeping in touch about.

However, the tone is a bit insular, a quality very much at variance with science's internationalism and with the outgoing character of Australian scientists. Why not have a regular feature from someone in the scientific world outside Australia? It also needs some decent correspondence columns — the journal comes out once a month so this would certainly be feasible. To do it justice, the first issue did remind the readership to get in touch, but later issues I saw provided no evidence that anyone was taking any notice beyond contributing the articles. Finally, it also seems to be taking itself rather too seriously. Wasn't it Bertrand Russell who said "One of the symptoms of an approaching nervous breakdown is the belief that one's work is terribly important"? I suppose this is true of the scientific community as well as of individuals. It is worth considering that one reason young people don't find science very appealing is that it does not come across as a barrel of laughs — Kylie Minogue and Jason Donovan playing the young Marie and Pierre Curie might be an interesting experiment in communication and one that this journal could perhaps suggest to Australian television. ☐

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Proliferating titles

P. H. Gallimore

Cancer Cells. Editor Paula Kiberstis. *Cold Spring Harbor Laboratory Press. 12/yr. US $180 (institutional), $55 (personal).*
Cancer Communications. Editor in chief Alan C. Sartorelli. *Pergamon. 6/yr. US $180 (institutional), $30 (personal).*
Genes, Chromosomes & Cancer. Editors in chief Felix Mitelman and Janet D. Rowley. *Wiley-Liss. 6/yr. US $90.*

THESE are three very different journals: the first consists almost exclusively of monthly reviews, the second is an open forum for short papers covering any aspect of the cancer research field and the third encompasses the specific area of cancer genetics from cytogenetics to DNA.

Cancer Cells is intended to cover all aspects of new developments, from basic science to clinical research. As the journal includes "commentaries, opinions, hypotheses and reviews", sometimes the scope and format of these articles overlap. Clearly one of the editors' aims is to create a more formalized version of a journal club and only time will tell if this goal is attainable in print.

I like the general layout, the uncomplicated manner of the illustrations and short but concise presentations. Most of the specialized cancer journals also publish short reviews. By virtue of its multidisciplinary nature, however, the field is vast and rapidly expanding; this new venture has succeeded in several novel ways in making a niche for itself. Meeting and book reviews, together with an extensive meetings and symposia calendar, are useful additions. For those who like to have a refresher course on the origins of oncogene and proto-oncogene acronyms each issue has an "oncogene file" in which one or two genes are defined, characteristics briefly summarized and a few key references provided.

Cancer Communications is a journal which aims to publish short research articles rapidly. The diverse spectrum of articles published so far makes this publication attractive only to research institutes or medical school libraries. There are a large number of established journals which are in direct competition with *Cancer Communications* and I think that this one will survive only if members of its illustrious editorial advisory board attract better quality science or submit a proportion of their own data for publication here.

Genes, Chromosomes and Cancer is also in a very competitive area, the competition coming not only from traditional cancer and genetics journals but from prestigious publications like *Cell*, *Science*, *Nature* and *Proceedings of the National Academy of Sciences*, to name but a few. Nevertheless, I think that this journal has got off to an excellent start by attracting good papers from most of the top laboratories in the field. The editors hoped that this would be a venue for rapid publications, but I note that some papers have taken 4–6 months following acceptance to get into print; this must be improved. The layout of this journal is neat but the quality of some of the illustrations showing banded chromosomes is barely acceptable. Some attention needs to be paid either to the primary submissions or to the quality of the journal's paper.

The realization of the number and diversity of genes involved in cancer and the complex nature of the cytogenetic changes associated with progression of the disease ensures a fertile field for this publication, at least in the short term.

The title of this review reflects my growing anxiety and frustration that the expansion of new titles in this field makes the task of keeping up with the literature an increasing problem. □

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Mixture for the market-place

D. Swirsky

Leukemia and Lymphoma. Editor in chief Aaron Polliack. *Scientific and Technical Book Services. 6 issues/volume. £34 (personal), Dfl.400 (library), Dfl.532 (company).*

PROBING the molecular events which culminate in haematopoietic malignancies is quite properly the leading edge of research on leukaemia and lymphoma. Such work is distributed through various established quality journals, both scientific and medical. *Leukemia and Lymphoma* does not profess to compete for readers on those terms, but aims fairly and squarely at "providing a journal for those practising physicians and scientists involved with laboratory and clinical diagnosis, clinical care and management and therapy of patients with leukemia and lymphoplasmacytic disorders". It is all too easy to forget that leukaemias and lymphomas are lethal diseases affecting 24 per 100,000 children and adults a year. Day-to-day decisions on the diagnosis and treatment of these conditions need to be as well informed and accurate as possible. We take it for granted that patients want the best available treatment. Physicians genuinely wish to provide it.

Leukemia and Lymphoma is setting out its stall in a very busy market-place. Competition in the field in ferocious — *Leukemia* and *Leukemia Research* are pitched directly at the same customers; *Blood*, *British Journal of Haematology*, the *European Journal of Haematology* and *Bone Marrow Transplantation* are general competitors. Add the cancer, cytogenetics and pathology journals, and the task of launching a new journal in this field falls into perspective. The problem will not be of too few papers to fill the journal (the "publish or perish" pressures will ensure a steady flow); rather, it will be to compete for the best quality papers.

The editors are drawn from 10 countries, with only the United Kingdom boasting two representatives. Their strategy in the first six months has been to provide two to four high quality reviews per issue, allowing wide-interest subjects to be placed alongside more specialized topics. Autologous bone-marrow transplantation in acute lymphoblastic leukaemia, interferon-α in chronic granulocytic leukaemia and multidrug resistance rub shoulders with two refreshing contributions on leukaemia in tropical Africa. The editors have contributed substantially to the first volume, a glance through volume 1 of *Blood*, published in 1946, revealing a prestigious precedent.

The original articles are equally mixed: oncogenes and DNA genotyping, clinical trials, cytogenetics, electron microscopy and monoclonal antibodies all get space. Epidemiology is a welcome addition — a case-control study linking small family size and glue exposure to non-Hodgkins lymphoma, and agricultural chemicals to acute lymphoblastic leukaemia in the north of England.

Given current financial strictures, will this journal sell? I hope so. At the moment it has something for everyone, but perhaps its best bet will be to create a specific niche in the border area between haematology, clinical pathology and histopathology. Given the recent advances in immunochemical, cytochemical and molecular methodology to characterize haematopoietic cells in blood, tissues and cultures, and technical advances in light, confocal and electron microscopy, subject matter is not lacking. The reputation of the editor in chief places him in a strong position to attract good papers in this area, and the strength of the editorial board should ensure the journal's future. □

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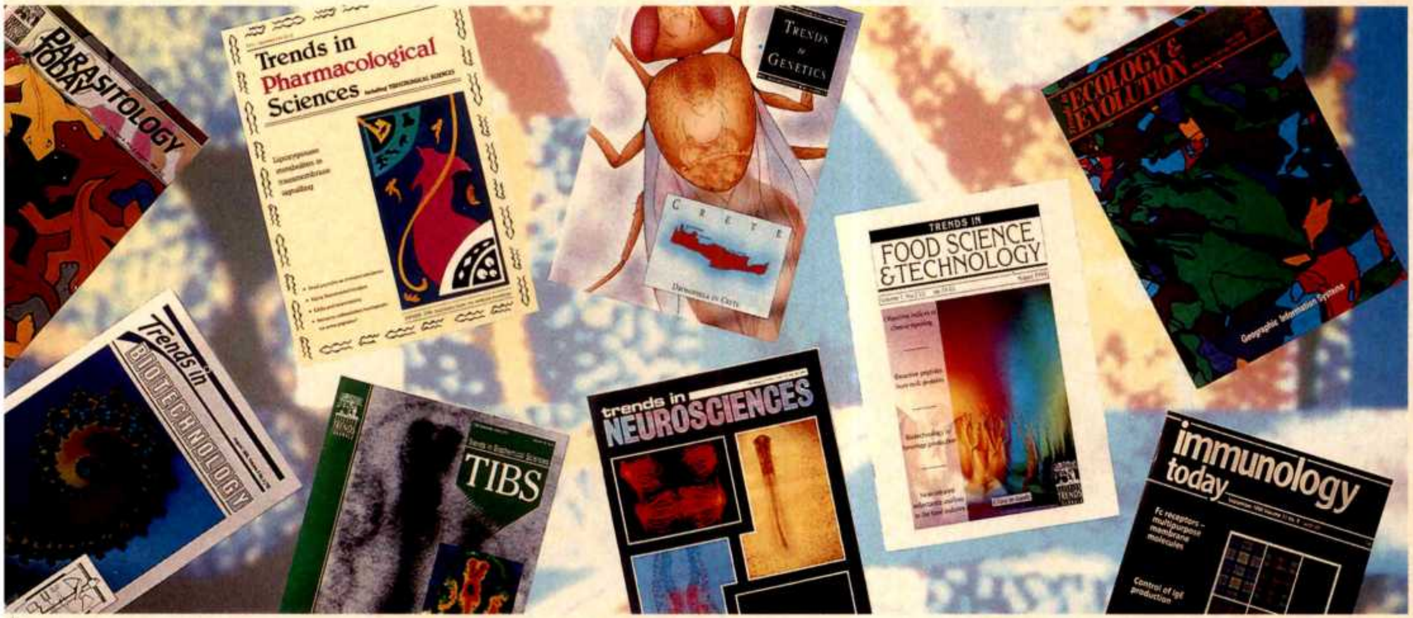

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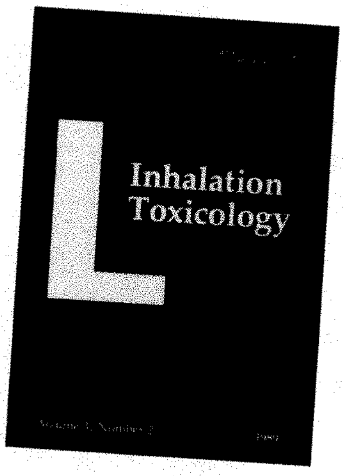
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Wide definition

K. R. Bruckdorfer

Journal of Lipid Mediators. Editor in chief B. B. Vargaftig. *Elsevier. 6/yr. US $210; elsewhere Dfl.373.*

AS THE boundaries between the classical scientific disciplines continue to erode and the number of publications increases, it is virtually inevitable that new specialist journals will appear, especially in areas identified as rapidly growing. It will be intriguing to see how the newcomers fare against the generalist journals in attracting the best-quality papers. The problem for the specialist journals is that contributors almost always study the particular specialism of the journal against another specialist background, and thus such contributors may sometimes have difficulty in deciding where to send their papers.

Journal of Lipid Mediators was launched in January 1989 and there are now about eight issues. The journal aims to carry articles on the chemistry, biophysics, biochemistry, pharmacology, toxicology, pathology, immunology and clinical aspects of lipid mediators. Lipid mediators are defined by the journal as lipid derivatives from all sources and include lipid-soluble vitamins, phospholipids, lipoproteins, lipopolysaccharides or fatty acids and their metabolites and derivatives. Synthetic lipids with biological activity are also considered. The definition seems to be surprisingly broad given the existence of some excellent journals of long standing that also cover the lipids–lipoproteins area, including at least one published by the same publisher as that of the new journal.

Analysis of the articles in the journal, however, shows a different picture. In the four volumes of this year, nine out of thirteen original articles concern platelet-activating factor. In another section, there are abstracts from the International Union of Pharmacology satellite conference on platelet-activating factor held in Paris in June of this year. The other articles are about diacylglycerols and phospholipase A_1, and there is a review of the effects of lipid mediators in inflammatory skin disease.

The articles are all of a high standard and from good laboratories. The apparent imbalance may reflect the interests of some of the editors and associate editors, who have a first class reputation in the platelet-activating-factor field. The editorial board, however, contains many eminent researchers from both European and North American laboratories and represents a much wider spectrum of interests — closer to that of the aims of the journal. It remains to be seen whether the journal will be able to broaden its base and attract more contributions in the arachidonate and eicosanoid area or to be a focus for discussion on post-receptor signalling. It would seem that the journal's scope has been cast too wide and that it will have little hope, for example, of attracting many of the main lipoprotein papers. But if the same standards are maintained and a crisper definition of a lipid mediator is made, there is every prospect that the journal will make a useful contribution to our knowledge about these important compounds. □

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Healing arts

Michael T. Lotze

Biotherapy. An International Journal on Biological Agents. Editor in chief Huub Schellekens. *Kluwer. 4/yr. Dfl. 332 (institutional), Dfl. 200 (personal).*
Molecular Biotherapy. Editor Robert K. Oldham. *Butterworths. 4/yr. North America $145; elsewhere $175.*

ONE of the fables that exists in the medical collective consciousness is the story of the young house officer who regaled one of his colleagues with the notion that he wasn't very good at diagnostics in approaching human diseases, but felt that he had mastered the intricacies of therapy. The dilemma of many individuals facing a person with cancer has been just the opposite: not so much the diagnosis, but the intractability of the disease in the many people (more than half of those diagnosed) with resistance to conventional modes of therapy (usually surgery, radiation therapy and chemotherapy). The precise goal of the biotherapist is in large part to disturb the homeostatic balance between the host and the tumour. The availability of various recombinant cytokines, monoclonal antibodies and cell populations for adoptive therapy has excited the imagination of scientists, clinicians and the lay public alike.

Two new journals, *Biotherapy* and *Molecular Biotherapy*, international in scope and format, attempt to provide a forum for individuals working within this area. Because the borders of biotherapy are somewhat indistinct, evaluation of these biological agents in various other settings, particularly those of infectious disease and haematology, represent areas of investigation included in articles in the first four issues of both journals. At the outset, the reader should also know that I am on the masthead of an older (*Biologic Response Modifiers*) and a newer journal (*European Cytokine Network*) which have scopes not dissimilar to the two I am reviewing here.

Attempting a rational programme of development from the dizzying array of biological agents available for laboratory, preclinical and clinical studies is a formidable task. In the early 1980s, the Biologic Response Modifiers Program of the National Institutes of Health was established in Frederick, Maryland, at Fort Dietrich in facilities reportedly previously used for evaluating germ warfare. Early studies were initiated and promoted under the leadership of Robert Oldham and Ronald Herbermann, and out of these arose the *Journal of Biologic Response Modifiers*. This journal was adopted by the Society of Biologic Therapy as its official publication and has entered a more mature phase, with monthly issues, in a larger format and with articles cited in the *Index Medicus*. Oldham, who initially steered this vehicle through its embryonic stages, has moved on to assume the helm of *Molecular Biotherapy*. This journal, as well as its counterpart, *Biotherapy: An International Journal in Biological Agents*, must be judged not only by their predecessors but also by their appearance and their promise for the future. Each of the journals is issued quarterly and has geographically placed editors, both in the Orient and the Occident. Both contain a series of articles of varying quality, typical of early start-ups that have not yet matured.

It isn't clear what format *Biotherapy* will eventually adopt. The earlier issues, starting in 1988, carried articles without subsection headings, ranging from the evaluation of recombinant cytokines in murine models of cancer and infectious disease to the role of Chinese herbal derivatives on superoxide scavenging, and the role of cholera toxin in inducing autoimmunity. Various reviews are included, discussing individual cytokines or areas as broad as biotechnology in Europe. The most recent issue I was sent for review includes articles focusing on the potential therapeutic uses of interleukin-1, edited by Charles Dinarello and Ruth Neta. This kind of assembled information provides a very useful review of the current status of this particular area and has significant advantages in reviewing this fast-moving field over textbooks, which have an obligatory one to three years of production delay. It is unclear whether such focused reviews, some of which are written by as many as 15 investigators, will become the exception or the rule in this journal.

Molecular Biotherapy is more differentiated in its organization: there are editorials and/or commentaries, largely by Oldham, a section entitled "review" and a series of papers. Patent reports, meeting reports and a calendar are variously included in different issues. "Commentary" provides a vehicle for Oldham to discuss his assessment of biotherapy and the means to test it in the clinical arena. Last year, interleukin-2 was approved for clinical use in Europe. This summer, the FDA approved interferon-γ for the treatment of chronic granulomatous disease, and provided an initial stay of approval of interleukin-2 for the treatment of renal cell carcinoma. The Recombinant DNA Advisory Committee also approved the first studies attempting to perform gene therapy, in which the genes encoding two "biologics", adenosine deaminase and tumour necrosis factor, will be used.

There are ample reasons to think that this field will expand and require the kind of forum provided by these journals. Much of the current excitement that pervades modern biological sciences, however, has developed because of the reductionistic approach taken to isolating and identifying the molecular species that appear to be the prime movers in biology. The introduction of molecules such as these, which have been so painstakingly dissected in recent years, back into the complex biological systems from which they arose, and to do so in a way which is formalistically and scientifically sound, represents both the problem and promise of the field and these new journals. Their success or failure in moving beyond their so-far inadequate contents will depend on their ability to apply the same rigorous standards as the more mature scientific disciplines (and journals). ☐

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Viable offspring

John Aitken

Molecular Reproduction and Development. Editor Ralph B. L. Gwatkin. *Wiley-Liss. 6/yr. US $100 (institutional), $50 (personal); elsewhere $116 (institutional), $66 (personal).*

Reproduction, Fertility and Development. Chairman of Editorial Board J. K. Findlay. *CSIRO. 4/yr. Australia and New Zealand $A120; elsewhere $US120.*

IT WOULD be difficult to imagine a more important area of biology than reproduction. A plethora of diverse global problems, including overpopulation, infertility, childbirth, HIV transmission, animal production and the preservation of endangered species, all fall under the umbrella of reproductive research and are dependent on advances in our fundamental understanding of this process.

Given its importance, it is perhaps surprising that reproductive biology, particularly the reproductive biology of mammals, has been such a neglected area of biomedical research. For much of the recent past, the subject has been dominated by endocrinologists who, while scoring a conspicuous success in providing the scientific basis for the contraceptive pill, have spent much of the intervening decades in an obsessive pursuit of hormonal profiles. The result has been a wealth of detailed technical data describing the vagaries of the hypophyseal gonadal axis in every sanguineous species from the aardvark to the zebra, but only a limited insight into the mechanisms regulating gonadal function.

A harsh generalization, perhaps, but it is undeniable that this whole-animal, hormone-orientated approach to reproductive research has, at last, reached its climacteric. Its place is gradually being taken by a more fundamental, molecular approach, in which emphasis is placed on the cellular mechanisms regulating reproduction, rather than a description of circulating hormone levels *per se*. These two new journals are intriguing in that they reflect this division in reproductive science between the traditional, endocrine-based approach and the influence of the recent intrusion of molecular and cellular biology into this area.

Molecular Reproduction and Development firmly declares itself in the latter category. There is an unfortunate fashion among new journals to seek respectability by including the adjective 'molecular' in the title; titles such as *Molecular Andrology* and *Molecular Endocrinology* abound and it can only be a matter of time before the *Journal of Molecular Sexual Practice* appears. The question is, then, to what extent is *Molecular Reproduction and Development* the genuine article? That it is can be deduced from the quality and international scope of its editorial board and the fact that Ralph Gwatkin, who previously created the excellent journal *Gamete Research* for Wiley-Liss, is its editor-in-chief. *Gamete Research* has been incorporated into the new journal, and as this area of reproductive research is, by its nature, dependent on a molecular, cellular approach, the fusion is both rational and effective. The papers I feel competent to judge seem to be of uniformly high quality and I have no doubt that this journal will fill an important niche in reproductive biology and, moreover, be important in shaping the future of this discipline.

I also hope that *Reproduction, Fertility and Development* will find a niche in the future landscape of reproductive biology. This Australian journal, with an all-Australian editorial board, is published by the Commonwealth Scientific and Industrial Research Organization of Australia and it contains, in the main, papers from Australian laboratories. It has an all-encompassing brief in that it solicits original articles, reviews and comments on all clinical and scientific aspects of reproductive and developmental biology as well as reproductive endocrinology, including relevant aspects of puberty and lactation. The subjects covered are extremely diverse, although many, if not most, of the contributions exhibit a traditional, comparative endocrinology lineage, characteristic of the well-established journals in this field such as the *Journal of Reproduction and Fertility* and *Biology of Reproduction*. Not surprisingly, however, there is an increased emphasis on marsupial reproduction in the Australian example.

A mouse embryo at 15 days of gestation (from *Molecular Biology of the Cell*, published by Garland).

Many of the papers are excellent, reflecting the fact that Australia has, in recent years, led the world in several key areas of reproductive research, most famously in *in vitro* fertilization but also in areas such as the endocrinology of inhibin. But some of the papers on other topics in the first few issues are less than vintage, and would have struggled to see the light of day in any of the mainstream reproduction journals.

Herein lies the dilemma. *Reproduction, Fertility and Development* could develop into a useful organ of communication between Australian laboratories, reflecting the unique interests of the local scientific community. But there is a danger that it will become a repository for second-class papers which either have been, or would have been, rejected by the main journals in the field. A stringent editorial policy will be needed if this journal is to retain its quality, appeal — and viability. ☐

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Watch this space

Eric A. Barnard

Journal of Molecular Neuroscience. Co-editors Paul J. Marangos and Julia M. Polak. *Birkhäuser. 4/yr. US $131; elsewhere $140 (surface mail), $161 (air mail).*

The number of journals with an emphasis on molecular neurobiology is now noticeably increasing. As this is probably the most rapidly growing field in the whole of present day biology, there is justification for this recent proliferation: for example, the editors of *Neuron* (established 1988) already state that they can now take only a relatively small fraction of the papers in that field which they deem meritorious.

There will surely be a growing demand for more space for this subject-matter for some time to come, if it is in a journal of high repute. I do not join those who lament (for example in a couple of last year's new journal reviews, of the sour grapes variety) over the recent multiplication of journals in this field, when it is at such a stage of exponential growth: it has yet to be demonstrated that the molecular neurobiology periodical market is anywhere near being well enough catered for. In the present state of excess demand, idiosyncratic or coterie editorial preferences appear in some places, and more competition is the only effective answer.

Reputation is necessarily built slowly by a new journal, and the only quick alternative is for it to start out as the offshoot of a long-established and well-regarded periodical machinery of a well-established publisher in that field. The *Journal of Molecular Neuroscience* did not start off with this silver spoon and Birkhäuser is not a household name in biomedical science publishing. So it has a long road to travel, and has had to begin in quarterly form and with an average of only seven papers per issue. It will need to grow to a fuller extent and to a monthly appearance before it can make a real impact.

It has a good prospect of growing to offer such real competition. The editorial board is widely based in subspecialities and hemispheric distributions, and there is a good balance between eminent experience and youthful enterprise. The papers which the journal has attracted so far are for the most part interesting and of good quality. They include a few of the archival type to be expected for inaugural contributions, for example a paper by 13 authors which simply documents, very soundly but with not too fascinating a conclusion, that mitochondrial DNA has no significance in the inheritance of Huntington's disease.

The editors state in the second issue of volume 1 that they wish "to incorporate brain protein biochemistry as a major part of this new journal" and they contrast this aim, rather scathingly, with "simply" publishing "sequence data and cloning results". Cloned sequences are, of course, now telling us a great deal of what is new in cellular neurobiology. Fortunately, the subject matter of the papers so far published shows that the large editorial advisory board is disregarding that dubious distinction. Its members certainly have the range and ability to make this, in due course, another journal of high standards and wide repute in molecular neurobiology. There is nothing so far to suggest that they will not achieve this, and neither potential contributors nor readers need fear that, if they succeed in bringing it up to the top class, there would then be too great a degree of competition in this field. This has the potential to be a journal for all neurobiologists to watch and to develop. ☐

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Good behaviour

A. Richard Green

Behavioural Pharmacology. Editor Paul Willner. *Clinical Neuroscience. 6 issues/volume. European Community £87.50 (institutional), £45 (personal); elsewhere £100/$185 (institutional), £55/$100 (personal).*

The list of journals encompassed by the broad heading of psychopharmacology grows ever longer, and some titles are struggling somewhat to obtain enough good submissions and therefore flourish. It might be argued, therefore, that this is not a good time to launch yet another. But in its first year, *Behavioural Pharmacology* seems to have attracted a reasonable number of quality papers, and this has to bode well for the future.

The question arises as to reasons for this early success, given that the journal is neither tied to a learned society (unlike, for example, *Journal of Psychopharmacology* or *Neuropsychopharmacology*) nor issued by one of the large international publishing houses. A glance at the editorial board suggests one explanation. It is pleasingly international, and although the editors are senior, all have clearly been chosen because they are still in the thick of research — not just because they are names which might look impressive inside the front cover. A second explanation is perhaps contained in the line printed under the title on the front cover: "an international forum in which behaviour and pharmacology receive equal attention". Many pharmacology and psychopharmacology journals do not seem to be appropriate for papers on physiological psychology, whereas journals on the latter subject often publish little on drug effects. In other words, a niche exists for this new publication.

Behavioural Pharmacology publishes original articles, reviews and commentaries, and the September 1989 issue contained abstracts from a workshop held under the auspices of the European Behavioural Pharmacology Society. The journal also accepts clinical papers, but few have appeared so far. Each issue is well produced, but the cover is old-fashioned and is not going to stand out on the library shelf and attract the casual browser. The journal does not print either submission or acceptance dates, so potential contributors cannot see how long it is likely to be before their work would appear in print.

In the first few issues there were a substantial number of research reports by members of the editorial board or members of their departments. Only time will tell whether other behavioural pharmacologists will submit their best work to this new journal, and it is only then that one will be able to say that this journal has a secure future as a major "international forum". Nevertheless, at present, the signs are promising. ☐

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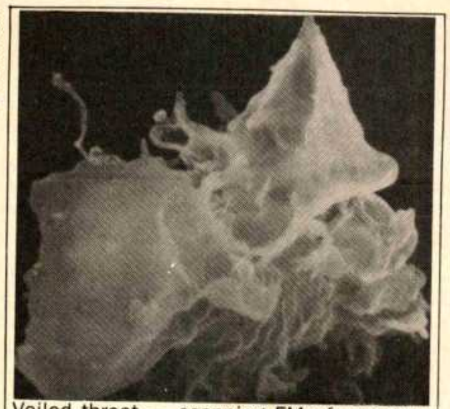
T cells or T shirts?

Peter Parham

Seminars in Immunology. Editor in chief Michael Julius. *Saunders. 6/yr. North America $110 (institutional), $60 (personal), $45 (student); elsewhere $130 (institutional) $80 (personal), $65 (student).*

Seminars *in Immunology* was a new one on me — perhaps because the stacks of Stanford's medical library toppled in last October's earthquake and order has yet to be restored. Its genre, however, is familiar. With this journal, one of a series "seminars in X", Saunders adds its contribution to the increasing selection of publications that distil the primary literature for those who create it. In immunology, these titles include *Immunology Today, Annual Review of Immunology, Advances in Immunology, Current Opinion in Immunology* and *Immunological Reviews*.

The flavour of *Seminars in Immunology* is most like *Immunological Reviews*, in that each issue is devoted to a single theme with relevant research being discussed by those who did it, or at least supervised its execution. Unlike *Immunological Reviews*,



G. MacPherson

Veiled threat — scanning EM of a mature dendrite cell showing two flattened lamellipodiae or 'veils'.

which is single-handedly edited by Göran Möller, each issue of *Seminars in Immunology* involves a guest editor — an internationally acclaimed guru in the particular field. This I discovered makes sense as, according to the *Oxford English Dictionary*, a seminar is "a select group of advanced students associated for special study and original research under the guidance of a professor". So far, the topics — T- and B-cell interactions, transgenic mice, T-cell development in the thymus and transmembrane signalling in lymphocytes — have covered active, rapidly moving areas of immunology, and the guest editors (respectively Charles Janeway, J.F.A.P. Miller, Jonathan Sprent and Gerry Klaus) have enrolled, with few exceptions, students of the highest calibre. Such "students" are not to be dominated by their professors and their essays are diverse both in style and scope, ranging from the overtly didactic to the demanding and impenetrable. In general they provide a snapshot of one laboratory's view of the universe it inhabits, and as such *Seminars in Immunology* is unlikely to benefit real students, new converts or the inquisitive heathen.

Most of these essays are full of meat, are frequently self-centred, and assume a familarity with the notorious vernacular that makes immunology suspect to other biologists. Moreover, there are enough typographical immunoballs to perturb and potentially disrail the uninitiated: an amusing example being Allison and Raulet's successive designation of "the other" type of T cells as gd, g/d and $\gamma\delta$, evoking a not implausible evolution from the righteous through the indecisive to the pagan. Other features that will deter teachers from using *Seminars in Immunology* are the tentative typeface, the predominance of uninspired computer-made line drawings, poor reproductions of photographs and the quaintly heraldic cover with its fake electron micrograph of a tubercular antibody molecule. The last would probably look good on a T-shirt.

With diminishing money available for their research, many immunologists are writing more and reading less. Numerous grants must be written as the chances of success for any given application decrease. These applications require bolstering with primary publications and, as these represent increasingly thinner slices of the salami, their significance and the insight to be obtained from reading them go down. Uncontrolled positive feedback between these two processes can set in, with the endpoint that once renaissance men and women become so enmeshed in writing their own grants and papers that they never read anything else. (This syndrome should be clearly distinguished from an older and highly esteemed condition, characterized by the voluntary preference to write rather than read the literature).

Saunders seems to appreciate this dilemma because *Seminars in Immunology* is tailor-made for those who find themselves snarled in its grasp. All the essays, irrespective of length, are heavily referenced — providing many useful citations, access to other reviews and entries into the primary literature, should one want to go that far. With each successive issue of *Seminars in Immunology* there has been an approximately linear increase in the mean number of references per essay (57, 59, 68, 72) and, with an annual ouput of six issues, I project that about 350 references per essay will be attained by the turn of the century. In addition, the essays themselves provide excellent workhorse citations as each permits reference to any result, idea or slip of the tongue emanating from a major immunology laboratory. On the other hand, there is risk for prospective authors in writing such essays, in that they will encourage others to reduce their oeuvre to a single citation. Such apprehensions can undoubtedly be overcome by the persuasion of an appropriately armed guest editor. In this regard it is of interest that, contrary to what one expects from a new scientific journal, only one of the eight members of the editorial board has contributed to the first four issues of *Seminars in Immunology*. ☐

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Risk and regulation

Francesco De Matteis

Chemical Speciation and Bioavailability. Associate editors Douglas Craft (US), Margaret E. Farago (UK) and T. M. Florence (Australia). *Science and Technology Letters. 4/yr. US $140 (institutional), $60 (personal); elsewhere £70 (institutional), £30 (personal).*

THE environmental impact of chemical pollutants such as potentially toxic metals is best appreciated by measuring the specific chemical forms in which the metal is present in the environment rather than its total concentration as, needless to say, different compounds of the same element may vary considerably in chemical and biological activity. The basic concept that different compounds have different behaviour is of course fundamental to all aspects of chemistry, including those pertaining to biological disciplines, such as biochemistry, pharmacology and toxicology, and applies equally well to environmental sciences. In practice, however, analytical difficulties in measuring specific chemical forms have often meant that in environmental studies, biological risk assessment and related regulatory aspects the chief emphasis has had to be placed on the total concentration of a toxic metal, rather than on trying to define and measure its potentially active forms.

The main purpose of this new journal is therefore to remedy the situation by encouraging research into the way in which the chemical form of potentially toxic metals influences their behaviour, including biological uptake and ecological fate. Contributions are encouraged from analytical chemists and environmental biologists alike, and also from scientists interested in engineering and regulatory aspects, with a view to providing a multidisciplinary forum for exchange of information in this area of applied research. The ultimate aim is to improve the chemical understanding of environmental issues and to provide more valid and scientific criteria for risk assessment to human health.

Although one can only agree with these general aims, time will tell whether, and to what extent, the journal will be successful in achieving them. The issues made available for review suggest that the editorial board needs to attract more high-quality papers for the journal to establish itself on a secure footing.

I am doubtful about the word "speciation" in the title: this word is rarely encountered in the literature (I myself had never met it before, neither had any of my colleagues); its meaning is not defined and I am not clear in what way it differs from the more commonly used word "species". As far as I can gather, it is meant to convey a variety of meanings — not only different forms of the same compound varying in oxidation state, charge, whether bound to an inert matrix, and so on. It is possible that "speciation" is a household word in environmental chemistry circles; if not, could it be defined in the first page of the journal? This suggestion has also been made by a member of the editorial board in a published letter to the editor. ☐

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NEW FROM CARFAX IN 1991

BIOCONTROL SCIENCE AND TECHNOLOGY

Editor: **C. C. Payne,** *AFRC Institute of Horticultural Research, East Malling, Maidstone, Kent ME19 6BJ, United Kingdom*

Supported by an International Editorial Advisory Board

Biocontrol Science and Technology is a new quarterly international bioscience journal to be published from March 1991. It will present original research and reviews in the fields of biological pest, disease and weed control. This will encompass basic scientific research, the application of new techniques such as genetic manipulation, and the technological development and application of biocontrol measures.

The Journal will cover:

• animal pest control by natural enemies (parasites, predators and pathogens) • biocontrol of plant diseases • weed biocontrol • 'classical' biocontrol • augmentative releases of natural enemies • quality control of beneficial organisms • microbial pesticides • properties of biocontrol agents; modes of action; methods of application • physiology and behaviour of biocontrol agents and their interaction with hosts • pest and natural enemy dynamics; simulation modelling • genetic improvement of natural enemies including genetic manipulation • natural enemy production, formulation, distribution and release methods • environmental impact studies; releases of selected and/or genetically-manipulated organisms; safety testing • the role of biocontrol methods in integrated crop protection • conservation and enhancement of natural enemy populations • effects of pesticides on biocontrol organisms • biocontrol legislation and policy; registration and commercialisation.

Research in this field is advancing on many fronts—in entomology, plant pathology, weed science, molecular biology, genetic engineering, ecology and production engineering. **Biocontrol Science and Technology** will provide a coherent focus for work from the broad range of disciplines.

Contributions to this journal are welcome. Copies of the Instructions to Authors can be obtained from the Editor. Tel: 0732 843833 Fax: 0732 849067

1991—Volume 1 (4 issues). ISSN 0958-3157.

NANOBIOLOGY
Journal of Research in Nanoscale Cellular Coherence

Editor-in-Chief: **Per Anders Hansson,** *CST Ltd, 67 Shakespeare Road, London W7 1LU, United Kingdom*

Co-Editors: **Hirokazo Hotani,** *Erato Molecular Dynamic Assembly, Kyoto, Japan;* **Stuart R. Hameroff,** *University of Arizona, Health Sciences Center, Tucson, AZ, USA*; **P. I. Lazarev,** *Institute of Biophysics, Academy of Science, Pushchino, USSR*

Nanobiology is a new quarterly international bioscience journal to be published from March 1991. It will present original research papers and topical reviews on all aspects of cellular systems in coherence. The journal's emphasis will be on processes acting on nanomechanisms such as repair, replication, and where the cytoskeleton acts as an operator. Within the scope of the journal manuscripts dealing with control from outside nanomechanisms are especially welcome.

Nanobiology will focus on engineering control at what has been called the 'hard-to-predict on the molecular level' force—i.e. electromagnetism. The journal will thus embrace experimental and theoretical work on both *nanorobots and nanoinformatics based on biological principles.* *Ethical issues* will only be considered when actual man-made products are within reach. Preliminary work-in-progress reports will be included, together with discussions of other research-community-strengthening activities.

Enzymes operating in coherence, such as in the endoplasmic reticulum and other subcellular structures, will be a main subject of coverage. Other topics will include:

• Cellular internal dynamics over time • Centrioles as nanoscale signal detectors • Coherent communicative resonance among protein conformational states and microtubular assemblies • Viruses as microreplicators • Microflora interfaces • Membranes as cooperative systems • Free radicals as nanosized control systems (i.e. triggering etc., not the biomedical long-term effects) • Electrical control of lipids • Engineering control of bio-organic molecular internal mechanisms

Contributions to this journal are welcome. Copies of the Instructions to Authors can be obtained from the Editor-in-Chief. Tel: 081 840 1082 Fax: 081 840 7776

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Covering cellular and molecular aspects of developmental immunology, this journal reports on both ontogenetic and phylogenetic issues, featuring contributions from experts in the field. The editorial board will be selecting high quality papers that serve to reinforce the journal's intended purpose - to keep you informed of expanding knowledge of developmental aspects of the immune system. If you wish to submit a paper for consideration, refer to the Notes for Contributors at the back of the journal.

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Editors: A. STIRLAND and T. WALDRON, Institute of Archaeology, London, UK

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Editor: C. THOMPSON, University of Southampton, Southampton, UK

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Editor: P. GRIFFITHS, Royal Free Hospital, School of Medicine, London, UK

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Editor-in-Chief: H.M. FRENCH, University of Ottawa, Canada

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Official Journal of the European Society for Medical Oncology

Annals of Oncology is a journal devoted to the rapid circulation of scientific communications in oncology. While the emphasis is on medical oncology, the input of basic research, surgery and radiotherapy is assured through representatives of these disciplines in the editorial office and board. The character of *Annals of Oncology* is multidisciplinary and also related to clinically oriented laboratory research, reflecting the expanding interest of oncology in Europe.

Subscription Information **ISSN** 0923–7534
1991, Volume 2 (10 issues)
Subscription rate: Dfl. 476.00/US$270.00

Inflammopharmacology

Inflammopharmacology publishes papers on all aspects of inflammation and its pharmacological control emphasizing comparisons of (a) different inflammatory states, and (b) the actions, therapeutic efficacy and safety of drugs employed in the treatment of inflammatory conditions. The comparative aspects of the types of inflammatory conditions include gastrointestinal disease (e.g. ulcerative colitis, Crohn's disease), parasitic diseases, toxicological manifestations of the effects of drugs and environmental agents, and arthritic conditions. There is no other single journal available at present which covers all the major aspects of the subject. The emphasis on comparative aspects of the actions of drugs is meant to highlight their efficacy and toxicity profiles as well as the variability in their clinical response and safety.

Subscription Information **ISSN** 0925–4692
1991, Volume 1 (4 issues)
Subscription rate: Dfl. 328.00/US$186.50

Geriatric Nephrology and Urology

The journal of *Geriatric Nephrology and Urology* will be devoted to the dissemination of information concerning the diagnosis and therapy of nephrologic and urologic disorders in elderly patients. This journal, which will include the broad range of clinical renal problems seen in the older patient, will stress the multidisciplinary approach necessary for the proper care of these patients.

Subscription Information **ISSN** 0924–8455
1991, volume 1–2 (6 issues)
Subscription rate: Dfl. 376.00/US$214.00

Cancer Therapy Update

Cancer Therapy Update, now in its tenth year, has provided critical commentaries on various aspects of cancer treatment. For this purpose, it has utilized editorials, timely overviews, case studies, nursing columns, and reviews of important journal articles (journal club). Its scope has included a striving for international cross-fertilization of therapeutic concepts and experience.

Subscription Information **ISSN** 0924–6533
1991, Volume 11 (6 issues)
Subscription rate Dfl. 135.00/US$73.50

Molecular Engineering

An Interdisciplinary Journal Devoted to the Design, Characterization, and Application of Molecular Materials Endowed with Specific Biological, Chemical, and Physical Properties

The journal *Molecular Engineering* is an interdisciplinary medium for the publication of original articles, interpretative reviews, notes and letters on the latest developments in molecular engineering. Articles reflect a predictive approach, based on well-defined structure-property relationships, aiming at the design, characterization, and application of organic molecules and molecular materials endowed with specific functions.

Subscription Information **ISSN** 0925–5125
1991, Volume 1 (4 issues)
Subscription rate: Dfl. 300.00/US$170.50

Bioseparation

International Journal of Separation Science in Biotechnology

Editor-in-Chief

Tony Atkinson, *PHLS Centre for Applied Microbiology & Research, UK*

Bioseparation publishes papers on all aspects of separation science applicable or potentially applicable to commercial-scale biotechnological processes.

Areas of particular interest include:

- recovery and purification of proteins, carbohydrates, antibiotics, biopolymers and other biomolecules.
- cell harvesting, cell disruption and primary separation
- recovery of extracellular enzymes
- process development and control, especially integration of operations and continuous processing
- phase partitioning, flocculation, centrifugation, filtration and precipitation
- adsorption, chromatography, membrane-based separations and electrophoresis
- developments and refinement of existing techniques
- applications of genetic engineering technology to bioseparation
- effects of cell physiology and compartmentalisation on separation
- economics of separation processes

Subscription Information **ISSN** 0923–179X
1991, Volume 2 (6 issues)
Subscription rate: Dfl. 512.00/US$290.50

Biodegradation

Biodegradation will publish papers on all aspects of science pertaining to the detoxification, recycling, amelioration or treatment of waste materials and pollutants by naturally-occurring microbial strains or associations or recombinant organisms. Areas of particular interest include:

– biochemistry of biodegradative pathways
– genetics of biodegradative organisms and the development of recombinant biodegrading organisms
– enhancement of naturally-occurring biodegradative properties
– applications of biodegradation and biotransformation technology, e.g. to sewage, heavy metals, organohalogens, high-COD wastes, straight-, branched-chain and aromatic hydrocarbons
– modelling and scale-up of laboratory processes and design of bioreactor systems
– international standardisation
– economic and legal aspects of biological treatment of waste

Subscription Information **ISSN** 0923–9820
1991, Volume 2 (4 issues)
Subscription rate: Dfl. 349.00/US$198.50

Environmental and Resource Economics

In cooperation with the European Association of Environmental and Resource Economists (EAERE)

The primary concern of *Environmental and Resource Economics* is the application of economic theory and economic methods to those environmental issues that require rational economic analysis (such as the greenhouse effect, transboundary pollution, deforestation, soil erosion, waste management, and noise).

Subscription Information **ISSN** 0924–6460
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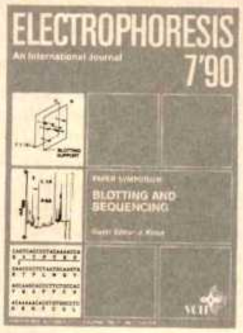

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Electrophoresis
Editor-in-Chief: Prof. B. J. Radola
Volume 12, 1991. Published monthly.
Annual subscription rate 1991:
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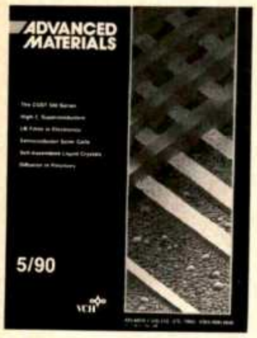
(International Edition in English)
Editor-in-Chief: P. Gölitz
Volume 30, 1991. Published monthly.
Annual subscription rate 1991: £ 290.00*



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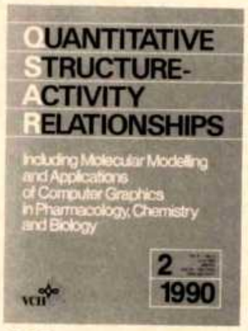
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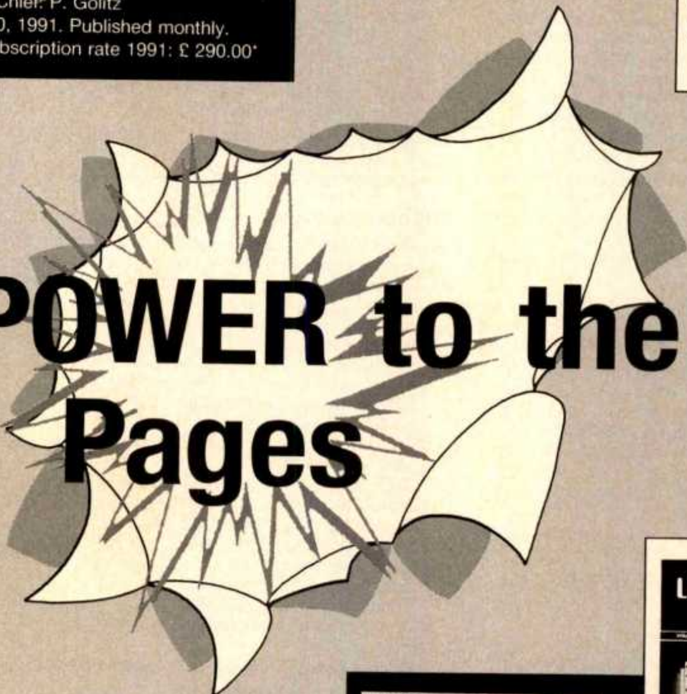

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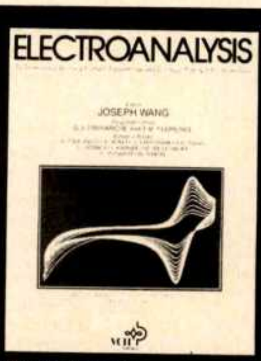
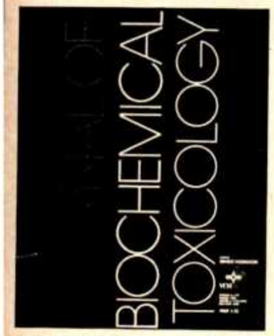
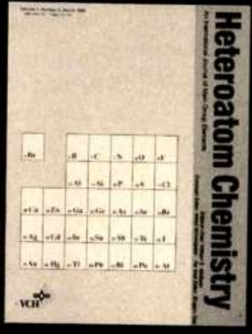


Electroanalysis
Editor-in-Chief: Prof. Joseph Wang
Volume 3, 1991. Annually 8 issues.
Annual subscription rate 1991: £ 192.0



Journal of Biochemical Toxicology
Editor: Ernest Hodgson
Volume 6, 1991. Published quarterly.
Annual subscription rate 1991: £ 120.00*

Laboratory Robotics and Automation
Editors-in-Chief: W.J. Hurst, J.W. Mortimer
Volume 3, 1991. Published bimonthly.
Annual subscription rate 1991: £ 110.00*



Heteroatom Chemistry
Editor-in-Chief: William E. McEwen
Volume 2, 1991. Published bimonthly.
Annual subscription rate 1991: £ 190.00*

Structural Chemistry
Editors: István Hargittai, Arthur Greenberg
Volume 2, 1991. Published bimonthly.
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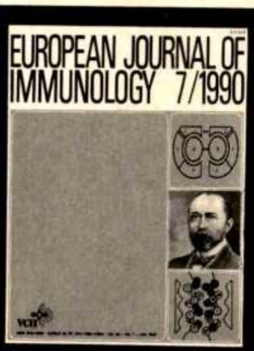

European Journal of Immunology
Editor-in-Chief: B. Kickhöfen
Volume 21, 1991. Published monthly.
Annual subscription rate 1991: £ 310.00*

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1991: Volume 28 (6 issues), Price: US$ 76.00/Dfl.152.00, including postage and handling. ISBN 0009-9325

The International Journal of RISK AND SAFETY IN MEDICINE

Side effects of Drugs - Devices - Surgery - Prevention - Liability.

Editor: **M.N.G Dukes,** *Univ. of Groningen, The Netherlands, and World Health Organisation, Copenhagen, Denmark.*

Risk and Safety in Medicine is an international journal for all those who are concerned with rendering the practice of medicine, surgery and the allied health professions as safe as it can reasonably be.
The question of risk management is rapidly becoming a major concern in the health care of the 90's. This is the first journal to address the field. It reports on and examines existing and potential risks, their causes, and means by which they may be reduced. Fields of special concern include: Risk management programmes in particular fields of medicine; Side effects and interactions of drugs, and the development of safer and more specific pharmaceuticals; Faults in medical and surgical practice; Quality assurance in medical equipment and materials; Legal liability, compensation and insurance.

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1991: Volume 5 (4 issues), Price: US $196.75 / Dfl.350.00, including postage and handling. ISSN 0269-2333

International Journal of ANTIMICROBIAL AGENTS

Editor-in-Chief: **Jan Verhoef,** *Department of Clinical Microbiology, University of Utrecht, Utrecht, The Netherlands*

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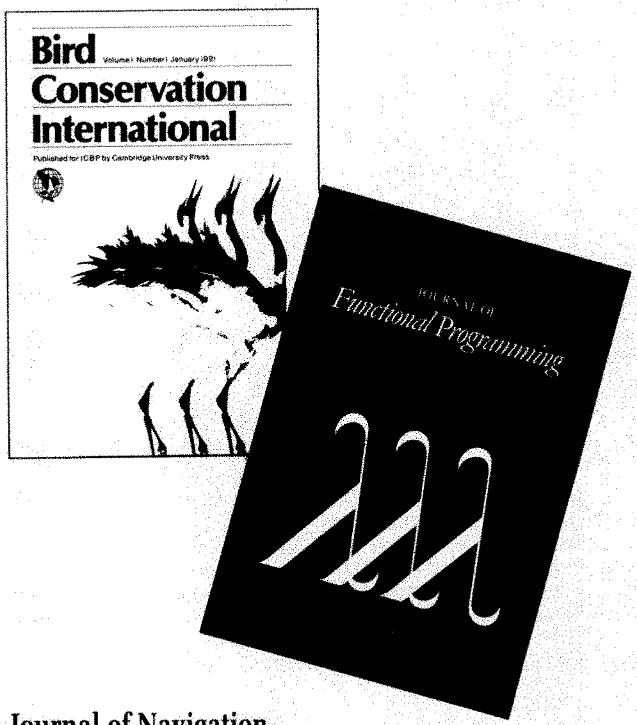
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Journal of Navigation

Comprehensive coverage of all aspects of navigation, including electronics, astronomy, mathematics, cartography, command and control, operational research, risk analysis, theoretical physics, operation in hostile environments, and instrumentation. From the Royal Institute of Navigation.

Volume 44: January, May and September 1991. £66 for UK subscribers; £71/$129 elsewhere; ISSN 0373-4633

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Political science

T. J. Cole

American Journal of Human Biology. Editor in chief Francis E. Johnston. *Wiley-Liss. 6/yr. US $90; elsewhere $114.*

THE start of modern human biology is generally dated to the growth studies of Buffon and de Montbeillard in the latter half of the eighteenth century. The journal *Human Biology* appeared in 1929, and in 1974 the British Society for the Study of Human Biology started its own *Annals of Human Biology*. Both journals have been consistently successful, but why in these straitened days should a new journal appear in this small niche of the market?

The answer, sadly, is less to do with science than with politics. In 1986 there was a disagreement between Frank Johnston, the incoming editor of *Human Biology*, and the publishers, Wayne State University Press, and this led the Human Biology Council, which had until then recognized *Human Biology* as its official publication, to take its custom elsewhere. The result is the *American Journal of Human Biology*.

The first issue appeared in January 1989, and there are to be six issues a year. The journal is printed to a high quality on glossy paper, though the fount is rather heavy for my taste. The format is conventional, with a dozen or so full-length articles plus book reviews. The Human Biology Council also publishes reports of its meetings. During 1989, four of the issues included symposia proceedings on growth and maturation; evolutionary, biosocial and cross-cultural perspectives on ageing; applications of molecular biology to issues in human biology; and influences of nutritional status on growth. These give a good idea of current research interests in the field.

Taking the fifth issue at random, the median time to publication was five months from acceptance and nine months from submission, perhaps slightly better than the competition. The quality of papers is generally very high, and it is clear from the authorship that several prolific research groups have switched their allegiances from *Human Biology*.

This raises the important question as to whether the market-place is big enough to support three journals in this field. *Human Biology* has tacitly answered this by announcing a switch in emphasis from general human biology to population biology and genetics. Judged by the first 18 months, the *American Journal of Human Biology* should continue to thrive, just as its predecessor has for 60 years. ☐

T. J. Cole is at the MRC Dunn Nutrition Unit, Downhams Lane, Milton Road, Cambridge CB4 1XJ, UK.

Pen and ink

Paul Fletcher and Philip Smith

Reading and Writing. An Interdisciplinary Journal. Editor R. Malatesha Joshi. *Kluwer. 4/yr. US $102.50 (institutional), $49 (personal); elsewhere Dfl. 210 (institutional), Dfl. 100 (personal).*

The write way — the approved writing position according to *Spencerian Key to Practical Penmanship* (America, *circa* 1860).

Mary Evans

READING and writing are areas of cognitive functioning of great importance to society. We would like to know more about how these skills develop, and what goes wrong in those individuals for whom they fail to develop adequately or who lose either of them in whole or in part. A new journal whose purpose is to publish research pertaining to the "processes, acquisition and loss of reading and writing skills" therefore deserves scrutiny. However, in the context of a very extensive literature, particularly on reading and spelling, published in the past 20 years in general psychological and educational journals as well as in specialist sources, a newcomer will have to work hard to establish an individual voice. A possible contribution, half-promised by the title, would be to give writing a research priority equivalent to that of reading. Writing (apart from its spelling component) remains a relatively mysterious and neglected process.

On the evidence of the early issues, this has not yet happened. Of the 25 articles in the issues we received for review, more than half concern reading only. Of the rest, only two are wholly concerned with writing. One of these, on backwards writing, may be thought to be addressed to a somewhat *recherché* skill. The other, which examines the grammatical deficiencies of learning-disabled students' writing, is of considerable potential interest, but could have benefited from an analytical framework somewhat more informed by developments in modern linguistics. *Reading and Writing* is subtitled "an interdisciplinary journal", and its editorial policy statement makes it clear that its purpose is to cut across the artificial boundaries that the different disciplines interested in literacy can erect. The early issues suggest that this is yet to be achieved.

The first volume certainly reflects recent work on reading: the remarkable recent expansion in reading research has arisen for various reasons, and the groups active in this area probably have very different aims in mind.

Cognitive psychologists have found written words ideal stimuli for studying perception, memory and attention. This is because the physical characteristics of words are readily specified, and many other properties of words, such as their frequency of occurrence, have been thoroughly recorded. Such research often uses reading as a means to an end, not as an end in itself, and the resulting research paradigms are not closely related to "normal" reading processes.

Cognitive neuropsychologists are interested in the functional architecture of the brain as revealed in brain-damaged patients, and have found written words convenient stimuli and responses, especially in patients whose communicative and cognitive abilities are limited. The most remarkable features of this group of subjects points to the modularity of reading processes: one patient (a deep dyslexic) might be able to guess the meaning of a word but not be able to read it out loud. Another patient (a surface dyslexic) might be able to read nonsense words aloud, but not real words (such as yacht) whose letter–sound correspondences are irregular. Yet another affected individual might be able to read only real words aloud, but not even the simplest non-word (this is referred to as phonological dyslexia). Observations like these have led to a view of reading as a skill requiring separate processes for converting writing to sound, accessing an internal lexicon, determining a word's meaning and so on.

A further source of stimulation has been the realization that there is more to writing and reading than the 26 letters of the English alphabet, and work on language and scripts as diverse as Chinese, Hebrew, Japanese and Serbo-Croat have significantly extended our knowledge of word-recognition processes. Technological innovations have provided a stimulus also: machines to read books aloud to the blind, or to recognize handwriting, and ways of monitoring eye movements or modifying text in response to eye movements have generated substantial amounts of research. ▶

Traditional interest in reading, in particular answers to such deceptively simple questions as why some children fail to learn to read, has perhaps become less prominent in the field as a whole, though even here, work on 'phonemic awareness' in preliterates and illiterates has given an impetus to certain types of teaching methods.

Apart from the use of reading to study perception, attention or memory, all these strands of research are represented in this new journal, though in differing degrees. The modularity hypothesis, in relation to normal and impaired subjects, in both reading and spelling, is the best represented. There is little so far on languages other than English, or that takes advantage of computational advances (although the computer-based real-time investigation of reading by Jarvella and colleagues is an excellent example of the genre). However, research in the 'traditional' questions of why children fail is surprisingly well-represented.

Although there is little sign yet, in the variety of topics, hypotheses and methodologies on show, of cross-disciplinary fertilization, it is fair to say that the first issues of this journal accurately reflect a diverse and thriving field. No doubt tighter integration will come, with a firm editorial hand. In the meantime, in these hard-edged times, there is surely something to be said for a journal catholic enough to find room to publish research on the effect of the study of Latin on spelling proficiency. □

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Casting neural nets

Stuart Sutherland

Neural Computation. Editor in chief Terrence Sejnowski. *MIT Press. 4/yr. US $110 (institutional), $55 (personal); elsewhere $124 (institutional), $59 (personal).*

JETAI : Journal of Experimental and Theoretical Artificial Intelligence. Editors Eric Dietrich and Chris Fields. *Taylor & Francis. 4/yr. US $120; elsewhere £65.*

WITH commendable restraint, Terry Sejnowski, the editor of *Neural Computation*, has refrained from writing an editorial for the journal's first issue. One can, however, infer from the contents that he intends to cast his neural net wide. There are theoretical contributions on new and — so the authors hope — improved parallel distributed processing systems: these have little or no direct bearing on brain function, though many of the authors end with the rather unconvincing claim that they are putting forward "plausible biological models". There are papers that purport to solve particular problems like inferring the three-dimensional structure of a scene from the shading of its surfaces: they rarely provide complete solutions and it is hard to say whether the ones proposed bear any relation to those taken by the brain. Finally, there are papers directly modelling the activity of real neurons and even ones on neuroanatomy, describing, for example, aspects of cortical connectivity.

The journal publishes lengthy and for the most part intelligible reviews of particular topics. In an era of increasing specialization, these are welcome: those on neural networks for speech recognition and for learning are particularly useful. The accounts of original research are deliberately limited in length and are, for no particular reason, called "letters", a nice compliment to *Nature*, which can — at least historically — justify the term.

Unfortunately, Sejnowski's misgivings about editorials are not shared by the editors of *JETAI* (a title stemming from the computing fraternity's belief that initials are more imposing than words). They view artificial intelligence as "a sober and circumspect scientific discipline — aware of its intellectual roots, presuppositions, and methods — that is at the same time, exciting, eclectic and justifiably optimistic". It is good to hear that things have changed, for in the past its protagonists have repeatedly been quite unjustifiably optimistic. Moreover, to be simultaneously sober and exciting is quite a *tour de force*. The journal covers all the topics included in *Neural Computing* as well as conventional AI and philosophical issues relating to the subject. It contains lengthy reviews as well as research papers, but many of the articles are vague prolegemena to research rather than reports of work actually accomplished. Several papers, for example, "On designing a visual system", tell the reader at unnecessary length what he already knows.

One wonders where the subject is going and how long it will take to get there. From a technological standpoint, the most useful programs, for example expert systems, are those that do not attempt to simulate the workings of the human mind, for people are notoriously bad at thinking. On the other hand, no program begins to rival the skills at which people excel, such as recognizing faces or carrying out a conversation at a cocktail party in a room full of echoes.

For the most part, conventional AI has so far merely helped to delineate the nature of certain cognitive tasks, to uncover the environmental regularities that constrain the interpretation of patterns of stimuli, and to reveal previously unrecognized problems, such as that of detecting the edges in a scene. Connectionism has different virtues. It has demonstrated that networks of neurons more or less randomly connected can show a hitherto unexpected capacity to learn quite complex tasks and can exhibit certain properties, like sloppiness, content-addressable memory and the ability to generalize, that characterize the human mind. But we are still a long way from knowing whether any of the many existing parallel distributed processing architectures corresponds to that used by the brain for a given task. Moreover, the proponents of parallel distributed processing ignore the fact that the brain is not simply a collection of neurons wired together in an excitatory or inhibitory fashion: it contains many neurotransmitters whose distribution is systematic and whose levels fluctuate.

Despite their use in clarifying our ideas, it is salutary to remember that so far both conventional AI and connectionism have only gone a small way towards the ultimate goal of understanding the human mind, but the humility one might expect this to induce is rarely displayed in either journal. Although the steps to understanding are small in both, those in *Neural Computing* are rather more solid. □

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Chipping in

D. J. Kinniment

Journal of VLSI Signal Processing. Editor in chief Earl E. Swartzlander, Jr. *Kluwer. 4/yr. US $173.50 (institutional), $75 (personal); elsewhere Dfl. 305 (institutional), Dfl. 180 (personal).*

International Journal of Computer Aided VLSI Design. Editor George W. Zobrist. *Ablex, 355 Chestnut Street, Northwood, New Jersey 07648. 4/yr. US $85 (institutional), $32.50 (personal); additional charge of $12 for overseas postage.*

IT HAS been said that electronic design techniques are being affected more and more by the black hole of VLSI, by which ever more of a system is sucked onto the surface of a single chip, never to return. These two journals are concerned with various aspects of designing on silicon, including the VLSI chip designs necessary to process speech or images (*Journal of VLSI Signal Processing*) and the tools and techniques involved in producing efficient and correct chips (*International Journal of Computer Aided VLSI Design*). In their mission statements, both journals make the point that VLSI design is a practical engineering discipline as well as one which bases its advances on theoretical work, and consequently many of the papers published here report on practical results and specific chip designs.

This is a useful approach, as it is often very difficult to compare widely different design techniques using different design tools for a range of applications, and publication of data on the results achieved is probably the best way of exposing the shortcomings of what seem to be promising theoretical ideas. A considerable proportion of the first year of publication of the *International Journal of Computer Aided VLSI Design* is dedicated to papers on the layout and interconnection of components on the chip surface. This is a problem to which well-known solutions exist, and where improvement, comparison and adaptation to new processing technology are the main areas of interest. Unfortunately this kind of article is read by only a few specialists, but a wider readership will welcome a special issue on VLSI computer-aided design in Japan which presents higher level work on a hardware description language, synthesis of circuits using knowledge-based techniques, and work on the effects of aberration in the optics used for defining the layer of masks used in chip manufacture.

The biggest problem now facing designers is the sheer size of the design task and the difficulty of getting from an overall specification, for example to reconstruct an image, to a detailed chip design of more than a million transistors, without making an error. There is, of course, no easy way of fixing errors once a chip is made, and there are no easy ways of checking its correctness fully before fabrication. Exciting developments in verification intended to address this problem are a hot topic, but are conspicuous by their absence in the first issues of this journal.

The material in the *Journal of VLSI Signal Processing* is of a high standard, with contributions from many of the well-known individuals and organizations in the field. Work on the fundamentals of arithmetic is represented as well as techniques for implementation of algorithms using systolic arrays, and actual chip designs. A new journal often has difficulties in maintaining a high quality of contributions while it is relatively unknown, and the approach taken here to maintain a good flow of publishable material is to devote the first two issues to extended and revised papers from the International Conference on Systolic Arrays held in San Diego in 1988. It will be interesting to see whether the editors can continue to attract the quantity and quality of papers necessary to sustain the journal in the future. □

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Hopeful recipe

John A. Campbell

Real-Time Systems. Editors in chief John A. Stankovic, Wolfgang Halang and Mario Tokoro. *Kluwer. 4/yr. North America $148 (institutional), $65 (personal); elsewhere Dfl. 311 (institutional), Dfl. 160 (personal).*

International Journal of High Speed Computing. Managing editors D. Gannon, A. Lichnewsky, Y. Muraoka and A. Sameh. *World Scientific. 4/yr. US $165; other rates on request from publisher.*

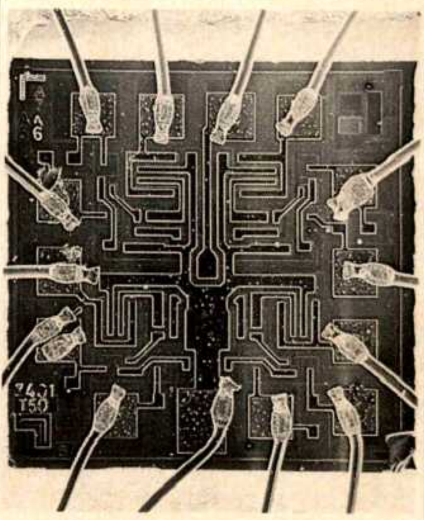
Impact of Computing in Science and Engineering. Managing editor Peter Deuflhard. *Academic. 4/yr. North America $80; elsewhere $92.*

Chance. New Directions for Statistics and Computing. Editors William F. Eddy and Stephen E. Fienberg. *Springer-Verlag. 4/yr. North America $50.95 (institutional), $26.95 (personal); elsewhere DM 121.70.*

THE financial arithmetic of producing a new and successful journal is much more attractive to a publisher than that of producing a successful research monograph. The only problem for a new journal is that in a time of increasing subscription costs and decreasing library budgets, how can enough paying customers be found to ensure success? To judge by the advertising material that I see, an important component of the ideal formula is to put computing into the recipe somewhere.

Publishers have now acted on this formula vigorously enough to give any good potential paper on computing a reasonable chance of seeing the light of day, in print, within a reasonable time. From being starved of journals only a short time ago, scientists with an interest in computing now tend to react to notices of each new one by asking (in the words of an anonymous mathematician who deserves better of posterity) which much-needed gap it is intended to fill.

One of the journals I review here clearly survives this test. *Real-Time Systems* does what its title suggests, which is to provide a welcome service in drawing together material that is otherwise not easy to find in one place. In addition, it emphasizes treatment of time-critical systems, and offers a home to several different subcultures which have not had much to do with each other in the past. For example, it contains papers by specialists in scheduling and time-dependent processes, who are now turning their attention to the distinctive problems of time-critical computing systems, and it recognizes that artificial intelligence also has a part to play in the subject, both as producer (of heuristics for scheduling, load-balancing and so on in applications too complex for exact methods) and consumer (of ideas that make it possible to estimate what resources and time are needed for a given computation to do its job — not a discipline that has been much in evidence in previous applied artificial intelligence).



Scanning EM of a silicon chip.

Dr Jeremy Burgess/Science Photo Lib

The future of real-time systems will mix all these strands together, and move the subject into the foreground of computer science. The editors' choice of papers indicates that they appreciate that database technology and communications also belong in this picture. Probably the only relevant topic that is under-represented at present is distributed systems, but this seems to be a random effect, given the size of the sample of papers in the issues sent for review. *Real-Time Systems* has a good chance of being the main focus in print for the future development of its subject; both developers and users stand to benefit from reading it.

One way to ensure that a journal gets a good supply of high-quality papers is to make explicit efforts to round up collections of material on particular topical issues, either by raiding stocks of well-refereed papers from research workshops or by commissioning specialists to act as guest editors for individual issues. *Real-Time Systems* has exploited this heuristic already. Another journal to do it, with positive results, is the *International Journal of High Speed Computing,* which covers behaviour, design and experiences of use of parallel computing, whether for supercomputers or special-purpose systems based on components such as the transputer. From my own point of view, the number of interesting and useful papers in the first volume is distinctly greater than average. But the fact that an average can be defined is a possible problem. The journal's scope is so wide that it has many competitors in each part of its coverage: various *IEEE Transactions* series, the *Journal of Parallel and Distributed Computing,* the *International Journal of Parallel Programming,* *Molecular Simulation,* and *Computer Physics Communications,* to name only those on my desk or in my department's reading room. Do we need another title? Probably not, though this opinion is consistent with the idea that the *International Journal of High Speed Computing* deserves to flourish at the expense of at least one of the journals just listed. I hope it maintains its first-volume standards and has the necessary luck.

The problem of competition will be even tougher in the case of *Impact of Computing in Science and Engineering.* The intention of this journal is to encourage communication among researchers and users of mathematical modelling, scientific computing and computer science, on the grounds that "the broad spectrum of techniques that are used nowadays to solve hard scientific and engineering problems is usually not represented within one journal". This is true, but the group of journals that covers the field (including titles mentioned above) tends to be displayed on adjacent shelves in good libraries: a reader interested in communication in print can find it just by moving his or her arms energetically while the feet remain fixed. A new journal in the area has to make its mark with papers that are, on average, more significant than those that its competitors carry. Unfortunately, there is no evidence from the first issues that *Impact* will do this.

Chance is the odd journal out in this selection. The best short description is that it is a trade and tribal quarterly for US statisticians. It follows a model that Springer-Verlag has used to good effect for similar subjects already — news, historical features, articles in popular style but with a reasonable amount of technical content, interactions with public policy — though somebody who is not a statistical professional in the United States may be a little intimidated by the amount of American knowledge required to make sense of the material.

Nevertheless, if one is prepared to allow some concessions over baseball and US politics, it is a consistently good read. The tribal spirit that it demonstrates is instructive, and well worth the attention of the type of anthropologist who treats quasi-scientific tribes like psychiatrists as fit objects for study. (This is not a joke — I think.) The *and Computing* in the subtitle seems more exciting than it is in practice: the journal mainly covers evaluation of statistical software for microcomputers. The subtitle was probably chosen because of the "computing sells journals" formula. But one can forgive much in a journal that carries a regular gastronomic column, provided that its columnist takes a more enterprising approach to selecting restaurants on his next visit to France. ☐

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Hand in hand

Robert D. Gillard

Tetrahedron: Asymmetry. Editor in chief S. G. Davies. *Pergamon. 12/yr. DM 550 (institutional), DM 153 (personal).*
Chirality. Editor in chief Irving W. Wainer. *Wiley-Liss. 4/yr. US $120; elsewhere $136.*

WHEN the Abbé Galiani said, a hundred-odd years ago, that "*Les dés de la Nature sont pipés*", he spoke of the difference already well known between natural and synthetic substances (dice, with their 1,2,3-spots clockwise or counter-clockwise are prototype handed objects). The first systematic attempt to discuss origins of the dominant handedness of natural molecules was Japp's lecture ("stereochemistry and vitalism") to the chemistry section of the British Association for the Advancement of Science in 1898. He concluded that asymmetry begets asymmetry.

This existence and influence of handedness in such natural molecules as amino acids, sugars, terpenes and alkaloids (and other drugs) has been a subject of interest and use for many years. The remarkable growth in research on handed molecules during the past 15 years or so has built on the efforts of the many scientists from Pasteur onwards, who had been fascinated by the diverse and diffuse phenomena of handedness.

These new journals (which will not lack for readers) recognize, in very different ways, this increasing activity in the synthesis, reactions and uses of handed molecules. Indeed, they are not alone: for example, the almost equally new-born *Journal of Molecular Recognition* (reviewed by Tom Blundell last year in *Nature* **341**, 357; 1989) also focuses on differences in such interactions as right–right versus right–left (or shaking hands as against holding hands). The UK Science and Engineering Research Council even has a special research initiative with the self-same title, molecular recognition.

Tetrahedron: *Asymmetry* is edited by S. G. Davies, who is at the Organic Chemistry Laboratory, Oxford University. Concerned chiefly with organic asymmetric synthesis and characterization of optically active molecules, it is (as one might expect from its *Tetrahedron* ancestry) directed towards organic — and perhaps organometallic — chemistry. This area is currently so active that it makes sense to provide a single focus for workers striving to increase optical purities of synthetic products. This objective has, of course, always been central to mainstream chemistry (and will certainly continue to be), and many organic chemists may think it a pity to separate it from general synthesis.

Chirality has three editors based in institutions concerned with pharmacology and the like. Its clear intention differs from that of *Tetrahedron*: *Asymmetry*: it is to make pharmaceutical and cognate scientists more aware of the importance of handedness of molecules. The word chirality (compare chiropody, chiropractor and chiromancy), due to Kelvin, is merely a more general description of handedness.

One cause of the upsurge in work on the handed molecules is the grim reminder given by thalidomide that effects on living beings of enantiomers often differ sharply: one hand of this molecule had the desired effects, but the other was apparently teratogenic. The wrong hand was not merely "isomeric ballast". Similarly, one hand of penicillamine is beneficial in the treatment of Wilson's disease of copper metabolism, whereas the enantiomer has unpleasant effects, and benoxaprofen (a racemic anti-inflammatory drug) was withdrawn in 1982. The Kefauver–Harris amendments to legislation in the United States arose from the same concern as that which has motivated the foundation of this journal. *Chirality* covers a novel and valuable area, though one or two papers do seem irrelevant to pharmacy and so rather out of place. It is well produced on quality gloss paper, very useful for weighing out materials.

At the most recent meeting of the British Association, the chemistry section again held a session on chirality. Professor Stirling handed out caraway seeds and spearmint chewing gum. These flavours, quite distinct to our taste detection system, itself handed, apparently stem from the enantiomers of a single terpene. That kind of observation underpins the fascination and usefulness of this field of handed molecular interactions manifest in so many areas of science and art. Both these journals will serve a useful function through their different perspectives on handedness.

I must raise two quibbles. First, both journals use "asymmetry" in their titles when (*pace* Pasteur) they mean "dissymmetry". Both contain papers describing molecules with plenty of symmetry, albeit axial rather than reflectional. Second, someone (preferably boards of editors of new journals) really must decide and tell us whether we are to use SI units or not. No British high-school pupil has heard of a kilocalorie in a science lesson for 10 or more years, yet both these journals use these units unexplained. ☐

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From hydrogen to uranium

Robert W. Cahn

Chemistry of Materials. Editor Leonard V. Interrante. *American Chemical Society. 6/yr. US $49 members, $299 non-members; elsewhere $54–62 members, $304–312 non-members.*

NO, nuclear weapons don't come into it. . . the heading is meant only to denote the catholic coverage of the periodic table intended by this new journal. Chemists in the United States have recognized that materials are widely regarded as an important object of scientific attention, and there are numerous signs that they want some of the action. Next month, the American Chemical Society is organizing a conference with the title *Frontiers of Chemistry: Materials by Design*, and a recent issue of the Materials Research Society's bulletin carries an interesting article on how undergraduate chemists can be won for materials science. A recent book edited by Mary Good, published by the American Chemical Society, popularizes biotechnology and materials science. This new journal is clearly part of this offensive.

The journal began publication in January 1989, and in the first issue of volume 2 the editor writes about his perception of the journal's role. It is to serve as a forum for fundamental research at the interface of materials science, chemistry and chemical engineering. Processing gets special mention, as it often and very properly does nowadays in the United States. The difficulty of getting enough papers on polymers is the only warning note; editors of many other broad materials journals can only echo "amen". A special function of the new journal is cited as being to give a home to materials-oriented papers too "chemistry-oriented" for most materials journals. The editor knows exactly what he is aiming at.

Some papers that seem of interest well beyond the confines of chemistry laboratories are on: the ultrasonic irradiation of Cu powder; use of tris(trimethylsilyl) arsine to prepare GaAs and InAs; short- and long-range order in β''-alumina containing Na and Eu; magnetic properties of nanometre-scale iron particles generated by iron atoms clustering in cold pentane; anodic electrosynthesis of CdTe thin films; surface characterization and depth-profiling study of electrodeposited Cr films; deep anisotropic etching of tapered channels in (110)-oriented Si; rare-earth ions adsorbed onto porous glass: luminescence as a characterizing tool; and preparation, purification and densification of ZnS powder from organometallics. Another treats polymeric precursors to boron nitride.

Even leaving aside those papers which speak mainly or exclusively to chemists, there is enough in these first two years' issues to show that there is such a thing as materials chemistry, and that the journal worthily represents it. It occupies a niche distinct from the competition, such as *Journal of Solid-State Chemistry* (with more of a focus on crystal structure and defects) and *Advanced Materials* (with more emphasis on review articles). ☐

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Merger matters

J. S. Rowlinson

Journal of Physics: Condensed Matter. Editor in chief R. A. Cowley. *Institute of Physics. 51/yr. North America $2,271; elsewhere £1,195.* One section, **Liquids**, editor J.-P. Hansen, is available by separate subscription. *12/yr. North America $271; elsewhere £142.*

TWO years ago, the Institute of Physics decided that the division between the *Journal of Physics C: Solid State Physics* and *F: Metal Physics* was becoming difficult to defend. Many papers could have been assigned to either section and the institute thought also that it might be failing to attract other papers in the field of condensed matter because they did not fit comfortably into either class, as traditionally defined. These newer areas are surface properties, polymers, liquids, liquid crystals and glasses. The institute's solution has been to amalgamate sections *C* and *F* to form the *Journal of Physics: Condensed Matter*, and to identify a part of their new journal as *Liquids Papers*. Professor J.-P. Hansen of Lyon, supported by a separate editorial board, is in charge of the new subsection.

The institute also took the more unusual step of extracting and publishing separately the papers on liquids, under the title *Liquids: a Section from Journal of Physics: Condensed Matter*. This subsection retains the original pagination. The parent journal appears weekly, with about 25 papers per issue, and the subsection is extracted monthly, with about 10 per issue.

The intellectual case for the amalgamation of sections *C* and *F*, and for the encouragement of the new borderline subjects, was clearly a strong one. The change has also been successful in practice; the papers published are numerous and of good quality. The liquids subsection has also got off to a good start, bolstered by some invited reviews on fashionable topics.

It remains to be seen how successful is the experiment of offering the liquids subsection both as part of the parent journal and as an independent journal. Almost all libraries will need the complete section on *Condensed Matter* if, indeed, they do not already subscribe to the whole of the *Journal of Physics*. But individual subscribers, tempted by the first year's offer of £59 for *Liquids*, will not have welcomed the price increase to £142 in 1990. ☐

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Extraction from the East

William Davenport and Paul Calvert

Metals Materials and Processes. Editor C. V. Sundaram. *Meshap Science Publishers, Lakshmi Building, Sir P. M. Road, Fort, Bombay 400 001. 4/yr. India, INR 1,500; elsewhere US $125.*

AS THE title implies, this quarterly journal covers a wide range of topics from metal-extraction processes through materials applications to materials modelling and theory. It contains a good mix of review articles and research papers, the latter mainly coming from Indian researchers. The journal is being launched at an important stage in India's technological development and many of the articles represent key aspects of this development. Papers on the behaviour of nuclear-reactor construction materials and fuels are, for example, given special prominence.

An important aspect of the journal is the emphasis it gives to materials processing. The core of materials science used to be seen as the relation between microstructure and properties. The new vision is that processing is a third main theme. What is not yet clear is how we can extract general principles from the details of specific methods for synthesis or shaping of materials. The editors acknowledge the importance of processing in obtaining enhanced materials properties and they are actively seeking papers in the processing area. Sol–gel processing and powder metallurgy metal-matrix composite processing are featured in the first year's issues. Papers on the thermochemistry of metal and metal compound production are also featured.

The first volume also includes papers on the extraction of primary aluminium and copper. These papers describe processing but seem to focus on improved methods of extraction rather than on methods of obtaining enhanced materials properties. They might more usefully appear in an extractive metallurgy journal.

The journal is dedicated to alloys, amorphous materials, ceramics, composites metals and polymers. All are represented, metals and alloys the most strongly. Polymers have not yet received the attention they deserve.

The journal is obviously in the hands of an energetic editor and editorial advisory board. It is a vigorous addition to the materials literature. ☐

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In from the cold

Arthur K. Covington

Electroanalysis. Editor in chief J. Wang. *VCH. 8/yr. US $250 (institutional), $95 (individual).*

ELECTROCHEMICAL science can broadly be divided into mechanistic studies of electrodes in solution and applications of the better-behaved systems to elicit information (analysis) or provide useful technological products (batteries, fuel cells and, perhaps, cold fusion). Although it might be said that those with research interests in electroanalytical chemistry need a new journal like a hole in the pocket, *Electroanalysis* must be welcomed. The first issue, published in January last year, contained some particularly interesting contributions and the standard seems to have been maintained in subsequent issues.

This year the number of issues has increased from six to eight. Special issues will also appear: the first (April 1990) was on the important new topic of microelectrodes and contained ten feature articles and three short communications; it is available separately at £24 (hardbound). These contributions appear to have been specially commissioned and are not part of a conference proceedings.

The format is large (nearly A4), double-column, and clearly printed, not camera-ready typescript. Publication from submission is reasonably fast at 6–8 months.

Electroanalytical methods of analysis and electrochemical sensors are clearly areas of increasing interest. Whether Wang and his distinguished international editorial board succeed in creaming away from established journals such as *Journal of Electroanalytical Chemistry* (if it recovers from publishing on 'cold fusion'!), *Electrochimica Acta*, *Analytical Chemistry*, *Analytica Chemica Acta*, *Selective Electrode Reviews* and the new divided *Sensors and Actuators Part B*, sufficient of the important material for *Electroanalysis* to maintain and even improve on its initial standard remains to be seen. I shall certainly have to include *Electroanalysis* in my personal scanning lists from its present performance. For individuals it is not expensive, at least not at the moment. ☐

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Too much heat?

Peter T. Landsberg

Continuum Mechanics and Thermodynamics. Editors in chief K. Hutter and I. Müller. *Springer-Verlag. 4/yr. DM. 298.*

THE aim of this journal is to bring together interesting applications of continuum mechanics and thermodynamics, bearing in mind the advances in recent years in non-newtonian fluids, liquid crystals, gas mixtures, shape memory alloys, and so on. In this task it appeals to engineers, physicists and mathematicians to send their contributions. There is also a distinguished international editorial board. The second volume has just started with papers received in September 1988 and April, May and July 1989, on topics such as heat and mass transport and waves in viscous fluids. On the available evidence, this will be a good quality journal.

I cannot, however, disguise my opposition to new journals unless they are absolutely essential. And this journal is not essential. The whole subject area is adequately covered by *Archive for Rational Mechanics and Analysis* (Springer), *Acta Mechanica* (Springer), *Liquid Crystals* (Taylor and Francis), the *Journal of Non-equilibrium Thermodynamics* (de Gruyter) and others, and also by general journals like the *Journal of Physics*, *Physical Review* and the *Proceedings of the Royal Society*. My university library, which has a considerable reputation, has already stopped taking journals essential for research, including old and well-established titles. There is no chance of it subscribing to this new journal.

Adding journals of this type, despite the distinction of their editorial boards, is a major disservice to the academic community which they claim to serve. One has to remind oneself of the question repeatedly raised in recent years — is the existence of many journals "for the benefit of the contributors only, not that of the readers"? (See John Merriman's article in last year's journals supplement in *Nature* **341**, 349; 1989.) ☐

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Making waves

Ian S. Robinson

Soviet Journal of Physical Oceanography. (A translation of the Russian language journal *Morskoi Gidrofizicheski Zhurnal.*) Editor in chief V. N. Eremeev. *VSP. 6/yr. DM 670, US $395.*

IT IS all too easy for English-speaking scientists to measure the progress of their subject in terms only of what is published in English. As the publication rate of scientific papers seems to accelerate relentlessly, it is not surprising that we often lack the stamina to keep abreast of recent work published in other languages, expecting the important results to be offered to English journals anyway. By adopting such an attitude we risk losing sight of the evolving trends of subjects which have a strong indigenous base in countries with their own scientific publishing culture. Such a case is the study of oceanography in the Soviet Union.

Physical oceanography has a strong history among Russian-speaking scientists, but despite attempts at East–West collaborative experiments, I fear that many of my colleagues share my ignorance of what is happening within Soviet marine science. At a time when physical oceanographers throughout the world are being called on to play a key role in improving our understanding of global climate change, and when political barriers are suddenly falling away, there is more reason than ever to foster communication between Western and Soviet ocean scientists.

These thoughts sprang to mind as I read through the first four issues of the *Soviet Journal of Physical Oceanography*, and discovered that Soviet oceanographers are concerned with very similar problems to their Western counterparts, but often offer a different perspective on their solution. For example, the authors of several papers examine the dynamics of mesoscale eddies and turbulence in the upper ocean, but the analytical and numerical approaches are rooted in the literature of Soviet applied mathematics. Experimental results are presented from observations in parts of the world's ocean which Western vessels rarely penetrate. Remotely sensed data come from more than the familiar NASA satellites. Thus, although I found nothing of outstanding scientific importance which would have been missed had this cover-to-cover translation of a Russian journal not been launched, I did find that it was the subtle differences in the way ideas are presented which stimulated new ways of thinking about our common oceanographic problems.

The extensive citation of other Soviet work also hints that there is much more to

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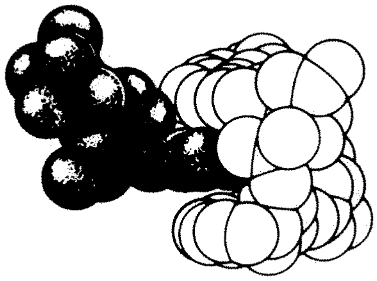

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The *Journal of Biomolecular NMR* provides a forum for publishing research on technical developments and innovative applications of nuclear magnetic resonance spectroscopy for the study of structure and dynamic properties of biopolymers in solution, liquid crystals, solids and mixed environments, e.g., attached to membranes. These studies may include:

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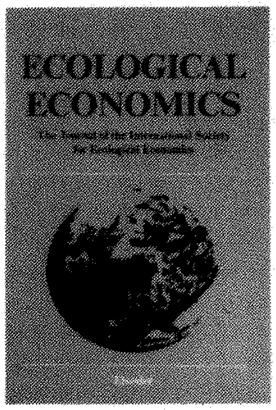
*************** *NEW JOURNALS* **************************** *NEW JOURNALS* ******

ECOLOGICAL ECONOMICS

The Journal of the International Society for Ecological Economics (ISEE)



Editor-in-Chief:
Robert Costanza,
Center for Environmental
& Estuarine Studies,
University of Maryland,
Solomons, MD, U.S.A.

Ecological Economics is concerned with extending and integrating the study and management of "nature's household" (ecology) and "mankind's household" (economics). This integration is necessary because conceptual and professional isolation have led to economic and environmental policies which are mutually destructive rather than reinforcing in the long term.
Specific research areas covered include: valuation of natural resources, sustainable agriculture and development, ecologically integrated technology, integrated ecologic-economic modelling at scales from local to regional to global, implications of thermodynamics for economics and ecology, renewable resource management and conservation, critical assessments of the basic assumptions underlying current economic and ecological paradigms and the implications of alternative assumptions, economic and ecological consequences of genetically engineered organisms and gene pool inventory and management.

Subscription Information:
1991: Volumes 3 and 4 (6 issues)
US$ 247.25 / Dfl. 440.00
ISSN 0921-8009
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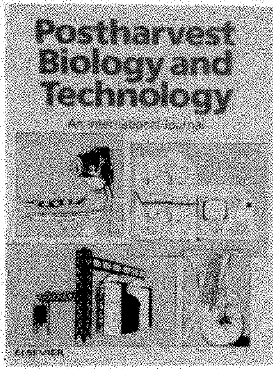
Postharvest Biology and Technology



Editors-in-Chief:
Graeme E. Hobson,
Institute of Horticultural
Research, Littlehampton,
West Sussex, U.K.,
Alley E. Watada,
Horticultural Crops Quality
Laboratory, USDA ARS,
Beltsville, MD, U.S.A.

This is the first international journal devoted exclusively to original papers and review articles o biological and technological research on postharvest handling or treatment, quality evaluation, packaging, storage and distribution/transportation of agronomic (including forage) and horticultural crops. Articles on the postharvest treatment of fresh product as affecting the quality of processed product will be included, but articles on food processing will not be considered for the journal. Papers based on interdisciplinary research will be encouraged. These disciplines include:

- ecology,	- molecular biology,
- entomology,	- chemistry,
- plant physiology,	- engineering,
- plant pathology,	- technology and economics.

Subscription Information:
1991: Volume 1 (4 issues):
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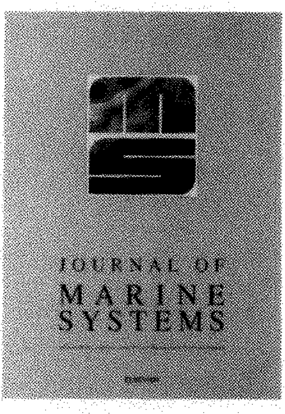
**************** NEW JOURNALS ****************************** NEW JOURNALS ***********

JOURNAL OF MARINE SYSTEMS

Journal of the European Association of Marine Sciences and Techniques

Editors:

K. Kremling,
Institut für Meereskunde, Universität Kiel, F.R.G.,
J. Nihoul,
GeoHydrodynamics and Environment Research (GHER), University of Liège, Belgium,
M. Vigneaux,
IGBA, Université de Bordeaux I, Talence, France



North American Editor:
G. Rowe,
Department of Oceanography, Texas A&M University, College Station, TX, U.S.A.

The purpose of the journal is to provide a medium of exchange for those engaged in marine research where there exists an interplay between geology, chemistry, biology, and physics. The Journal of Marine Systems welcomes research papers, review papers, short communications, discussions, book reviews and announcements on all aspects of marine sciences. Preference is given to papers which extend beyond the limit of a simple discipline and which follow the rationale of system analysis.

Subscription Information:
1991: Volume 2 (4 issues)
US$ 184.25 / Dfl. 328.00
ISSN 0924-7963
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EUROPEAN WATER POLLUTION CONTROL

EWPCA

Official Publication of the European Water Pollution Control Association

Editor-in-Chief:
J. de Jong,
Rijkswaterstaat, Institute for Inland Water Management and Waste Water Treatment, The Netherlands

In 1991 **European Water Pollution Control** will be launched by Elsevier Science Publishers on behalf of the European Water Pollution Control Association (EWPCA). The Journal will provide a focal point for communication between all European technologists and researchers concerned with the following and other related issues:

- collection, transport, treatment and disposal of sewage and waste
- sewerage systems; design, construction, maintenance
- prevention of water pollution
- urban stormwater collection, treatment and effects upon receiving waters
- treatment, use and disposal of sludge
- river basin management
- operation, management and staff training
- National, European and International directives, CEN-information, legal aspects

Subscription Information:
1991: Volume 1 (6 issues)
US$ 131.00 / Dfl. 233.00
ISSN 0925-5060
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NEW JOURNALS FROM PERGAMON IN THE PHYSICAL SCIENCES

Engineering, Materials Science & Energy

BIO-MEDICAL MATERIALS AND ENGINEERING

An International Journal

(Formerly Journal of Bio-Medical-Mechanical Behavior, Materials and Engineering)

1991: Volume 1 (4 issues) (959)

An interdisciplinary journal publishing original research papers, review articles and brief notes on materials and engineering for biological and medical systems.

Keywords: Bio-medical materials, bioengineering.

CHAOS, SOLITONS AND FRACTALS

Applications in Science and Engineering

1991: Volume 1 (6 issues) (967)

It is the aim of *Chaos, Solitons and Fractals* to provide a medium for the rapid publication of full length original papers, short communications, reviews and tutorial articles on the following subjects:

- bifurcation and singularity theory, deterministic chaos and fractals.
- stability theory, soliton and coherent phenomena.
- formation of pattern, evolution and complexity theory.

The emphasis of the journal will be on applications in the fields of physical sciences, biomedical and life sciences and engineering.

Keywords: Bifurcation and singularity theory, deterministic chaos and fractals, stability theory, soliton and coherent phenomena.

COMPUTER OPTICS

1991: Volume 3 (4 issues) (912)

Computer Optics is not just about computers in optics but also explores optics in computers. A host of optical elements already in production are being applied in information processing, and in solving a whole series of interesting problems.

Computer Optics is intended for a wide circle of scientists and specialists in optics, computer technology, quantum electronics and radiophysics.

Keywords: Optics, quantum electronics, diffraction theory, data visualization, information processing.

COMPUTING SYSTEMS IN ENGINEERING

1991: Volume 2 (6 issues) (916)

Communicates recent and projected advances in computational technology in engineering fields. Includes hardware, software, models and strategies, and applications to problems of analysis and design.

Keywords: Numerical simulations, numerical algorithms, computational strategies.

ENGINEERING APPLICATIONS OF ARTIFICIAL INTELLIGENCE

1991: Volume 4 (6 issues) (975)

This journal, grounded in the practical, inter-disciplinary world of the engineer, provides a forum for the transfer of knowledge in the rapidly-developing field of artificial intelligence.

Keywords: Expert systems, CAD and CAM, signal processing, industrial, production, chemical, civil and electrical engineering, AI programming languages, computer vision, robotics.

EXPERT SYSTEMS WITH APPLICATIONS

An International Journal

1991: Volume 2 (4 issues) (939)

This journal will focus on the exchange of information relating to expert systems applied in industry, government, and universities worldwide. The articles will discuss the design, development, testing, implementation, and/or management of expert systems, and also provide practical guidelines in the development and management of these systems. Of interest to engineers, developers, researchers, scientists and consultants involved with artificial intelligence.

Keywords: Intelligent control, intelligent systems, real-time AI, applications of AI.

MECHATRONICS

Mechanics . Electronics . Control

1991: Volume 1 (4 issues) (933)

Mechatronics is the much expanding field of engineering which integrates mechanical and electronic principles. The journal seeks to publish research progress in this field with an emphasis on the applied rather than the purely theoretical, it will also serve the dual role of bringing greater recognition to this important area of engineering.

Keywords: Mechatronics, electromechanical design, computer-integrated systems.

MICROGRAVITY QUARTERLY

1991: Volume 1 (4 issues) (950)

Aims to promote and diffuse the culture of the microgravity environment by addressing the multiple problems (scientific, technological, industrial, artistic, socio-economic, legal managerial) connected with the attainment of, permanence in, observation, study, harmless colonization and peaceful utilization of the Fourth Environment of Mankind.

Keywords: Microgravity, life, fluid, materials and engineering sciences.

RENEWABLE ENERGY

An International Journal Incorporating Solar and Wind Technology

1991: Volume 1 (6 issues) (969)

The journal seeks to promote and disseminate knowledge of the various topics and technologies of renewable energy and is therefore aimed at assisting researchers, economists, manufacturers, world agencies and societies to keep abreast of new developments in their specialist fields and to unite in finding alternative energy solutions to current issues such as the greenhouse effect and the depletion of the ozone layer.

Keywords: Solar energy, conservation, wind energy.

WELDING ABSTRACTS

1991: Volume 4 (12 issues) (934)

Over 500 abstracts are produced each month, written especially for the needs of the welding community. The abstracts are added to WELDASEARCH, the welding institute's database, available online exclusively via ORBIT Search Service.

Keywords: Welding, soldering, spraying (thermal), brazing, cutting (thermal), metallurgy.

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Physical Sciences

THE ASTRONOMY QUARTERLY

1991: Volume 8 (4 issues) (938)

Examines the role of astronomy, with an emphasis on cosmology, in natural philosophy and in culture in general, as well as astronomy's influence upon the intellectual atmosphere of the epoch. *The Astronomy Quarterly* features in-depth, non-technical articles written by professional scientists for the educated layman and student.

Keywords: Astronomy, cosmology, astrophysics, archaeo-astronomy, ethno-astronomy, paleo-astronomy, socio-astronomy.

ATMOSPHERIC ENVIRONMENT

Part B: Urban Atmosphere

1991: Volume 25B (3 issues) (848)

Published as a separate volume of *Atmospheric Environment*. Articles will include issues on field studies, data analysis, models and reviews of the effects of urbanization on the atmosphere. Part B is included as part of the subscription to Part A or may be ordered separately.

Keywords: Dispersion, transformation, micrometeorology.

BIO-ORGANIC & MEDICINAL CHEMISTRY LETTERS

1991: Volume 1 (12 issues) (972)

Presents preliminary experimental or theoretical research results, on all aspects of science at the interface of chemistry and biology, and on major advances in drug, design and development. The journal will publish preliminary communications in camera-ready form in much the same format as *Tetrahedron Letters*.

Keywords: Organic chemistry, medicinal chemistry.

QUATERNARY INTERNATIONAL

The Journal of the International Union of Quaternary Research

1991: Volumes 5 - 8 (4 volumes) (865)

A high quality scientific publication under the auspices of the premier Quaternary Association, which will reflect the interdisciplinary nature of INQUA and records recent advances in Quaternary Science that appeal to a wide audience. Will publish collected research papers from symposia, workshops and meetings sponsored by INQUA's Commission, Sub-Commission and working groups.

Keywords: Quaternary, geochronology, holocene, stratigraphy, fossils.

TETRAHEDRON: ASYMMETRY

1991: Volume 2 (12 issues) (937)

Tetrahedron: Asymmetry publishes experimental or theoretical research results of outstanding significance and timeliness on asymmetry in organic, inorganic, organometallic and physical chemistry, as well as its application to related discipines, especially bio-organic chemistry. Topics include the physico-chemical and biological properties of enantiomers, asymmetric synthesis, resolution, chirality recognition, analytical techniques for determination of absolute configuration, and molecular graphics and modelling methods.

Keywords: Asymmetry, stereochemistry, chirality.

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Editor-in-Chief : Gerald W. Hart, Johns Hopkins Medical School, Baltimore, U.S.A.

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Journal of Logic and Computation

Editor-in-Chief : D. M. Gabbay, Imperial College, London, U.K.

Logic has found applications in virtually all aspects of Information Technology, from software engineering and hardware to programming and artificial intelligence. Indeed, logic, artificial intelligence and theoretical computing are influencing each other to the extent that a new interdisciplinary area of *Logic and Computation* is emerging. The journal covers among others the following areas of interest : logical systems, constructive logic, categorical logic, modal logic, type theory, feasible maths; logical issues in logic programming, knowledge based systems and automated reasoning; logical issues in knowledge representation such as nonmonotonic reasoning and systems of knowledge and belief; logics and semantics of programming; specification and verification of programs and systems; applications of logic in hardware and VLSI.

The European Journal of Neuroscience

Editor-in-Chief : R. W. Guillery, University of Oxford

Now published monthly this important journal is an exciting initiative undertaken by the European Neuroscience Association. Of broad scope, ranging from the behavioral to the molecular it provides timely and topical papers of interest to all researchers. The Editorial Board represents all major areas of neuroscience and is truly international, guaranteeing papers of quality and relevance.

Journal of Neuroendocrinology

Editor-in-Chief : Stafford Lightman, Charing Cross and Westminster Medical School, London, U.K.

The **Journal of Neuroendocrinology** publishes timely papers dealing with the newest ideas, knowledge and technology in the area. Covering molecular biology, cell biology , biochemical studies, developmental studies, molecular embryology and genetics, general systems, receptor and messenger systems, electrophysiology, ion channels etc., clinical and anatomical aspects, it is now widely acknowledged as an essential new forum and resource.

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be discovered in fields such as ocean optics and acoustics. It is a pity that the reference lists do not consistently denote whether the cited papers are in English or Russian. Another shortcoming was the absence of a date of either submission or first publication in Russian, so that the reader cannot judge how recent the results are. The most recent citations are a few years old, and the release of the first four issues of volume 1 has itself spanned nearly four years.

The subject matter does indeed cover all aspects of physical oceanography, theoretical and experimental, small and large scale, computational and analytical, remote-sensing and conventional ship methodology. The papers are generally brief, written in a terse style which selects the key ideas and conclusions rather than presenting methods in detail, although not as abbreviated as some other translations of Russian marine journals. Some papers are written in lucid English, although the quality of translation is patchy. Thus the journal would not be very accessible to those new in the field of study, but for physical oceanographers this is a welcome newcomer which will surely repay regular inspection. ☐

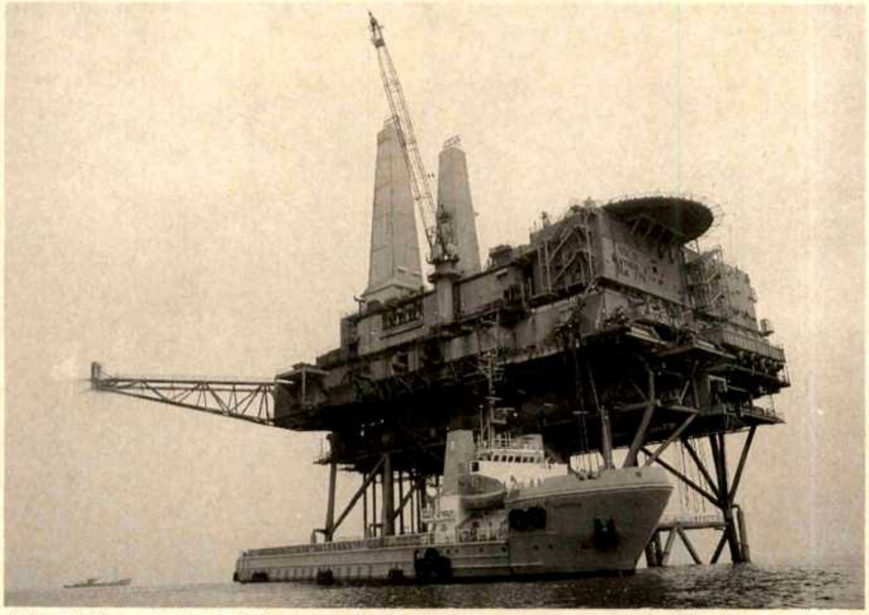
Ian S. Robinson is in the Department of Oceanography, The University, Southampton SO9 5NH, UK.

Insiders' viewpoint

Andrew S. Mackenzie

Oilfield Review. Executive Editor Henry N. Edmundson. *Schlumberger/Elsevier. 4/yr. US $128.50, Dfl.257.*

OIL companies have relinquished control of many technologies important for the location and exploitation of oilfields to subcontractors. The subcontractors are responsible for innovation — and the oil companies are simply informed consumers. The economies of scale available to subcontractors who provide these services allow them to outperform the in-house facilities of large oil companies. The more successful subcontractors have swallowed others to create large multinational service companies which assist oil companies from discovery to production.



A fixed oil production platform in the North Sea, owned by Union Oil, with supply ship alongside and safety boat in the distance.

Richard Folwell/Science Photo Library

Schlumberger — the highly renowned multinational service company — is the publisher of *Oilfield Review*, with Elsevier organizing printing and distribution. *Oilfield Review* is a demonstration of Schlumberger's involvement in the progress of many petroleum sciences.

The articles already published cover a stimulating range, from exploratory geophysics to corrosion control. They are all well illustrated, and readable by petroleum scientists from different specialist disciplines to those of the authors of the articles. Punchy summaries allow quick selection of material of interest. The level of coverage is designed for managers of petroleum science required to keep in touch with new advances involving all disciplines of petroleum science and engineering — or for specialists wanting to broaden their horizons.

This journal is not just written by and for different kinds of specialists who never talk to each other. There are several articles about work that involves different specialists working together productively on a problem which needs different insights, such as the evaluation of prospects offshore from Norway. Others address phenomena important to all petroleum sciences from seismics to reservoir engineering, such as the anisotropy of sedimentary rocks.

The journal does smack slightly of a Schlumberger advert — almost all contributors are company employees. This one company surely cannot claim to monopolize all the good ideas. It needs to invite contributions other than the guest editorial from outsiders — the subcontractor must learn to subcontract. ☐

Andrew S. Mackenzie is with BP International Ltd, Britannic House, Moor Lane, London EC2Y 9BU, UK.

Solid feature

Alec Smith

Terra Nova. Editor in chief R. Muir Wood. *Blackwell Scientific. 6/yr. North America $224.50; UK £120; elsewhere £135.*

TERRA Nova is a geosciences journal with a difference, if only for the breadth of coverage in every issue. Spawned by the European Union of Geosciences, the only pan-European society for all those interested in the sciences of the solid Earth, *Terra Nova* goes to all union members as part of its annual subscription of £30. With more than 100 pages per issue, they get a bargain. (Non-member and institutional subscribers have to pay considerably more.) Though the union started in 1981, *Terra Nova* made its first appearance in 1989 and is issued bi-monthly.

Terra Nova is much concerned with European geosciences. It has its eye firmly on the integrated European Community of 1992 and beyond into the new post-cold war Europe from the Atlantic to the Urals, and a fair amount of the Middle East as well. Published entirely in English, it offers speedy publication of refereed research papers in all aspects of the geosciences (the list would be too long to reproduce here) as well as regular sections of news, reviews, correspondence, books, computing, expeditions, eyewitness accounts, events and a calendar of meetings. The pattern is repeated in each issue and subscribers can always look forward to features with anticipation.

The journal is not for those interested in only their own chosen field or part of a field of geoscience: the approach is broad yet challenging. It is not a journal for the layman, but it is worthy reading for non-geoscientists who wish to see what the subject is currently about. For geoscientists, it offers a stimulating insight into progress in the entire spectrum of the subject together with a fair discussion of the field's pan-European political and social responsibilities.

One cannot fail to be impressed by the quality of the publication; not only by the depth of treatment but by the many topics already covered in the journal's brief existence. Two thematic issues have appeared in the eight issues to date: "Milankovitch cycles" (14 articles) and "Environmental geology and the marine dimension" (6 articles). Other splendid articles delighted me: the "Geology of the Bible" with many fine coloured plates and the well-merited reprint of Peter Gould's thought-provoking article "Tracing Chernobyl's Fallout" are but two of them.

This is a well-illustrated journal with many line figures and photographs. A particular feature (a house style?) is the use of reproductions of mediaeval woodcuts and nineteenth-century pictures, both as section markers and to illustrate the text where appropriate. This novelty may well wear off with time, however.

Much of the dynamism of *Terra Nova* owes a great deal to its editor in chief, Robert Muir Wood: his editorials are direct and thought-provoking (see, for example, his piece on the refereeing system and peer review) and the balance of each issue has been splendid. The journal sets out to achieve, and seemingly has succeeded, in making geoscientists communicate on more than what all too often are their narrow and personal interests while ensuring a rapid publication of research papers. The geographical spread of authors and frequent multinational co-authorship of papers to date certainly reflect the pan-European and broader appeal while the wealth of subjects reveal the breadth of geosciences today.

Since more than 2,000 participants attend each biennial European Union of Geosciences meeting in Strasbourg, *Terra Nova* should be assured of a sound subscription base. It deserves to succeed, for it will do much good for European research as well as for all those who care to read its papers. ☐

Alec Smith is in the Geology Department, Royal Holloway and Bedford New College, University of London, Egham, Surrey TW20 0EX, UK.

Icing on the cake

John Splettstoesser

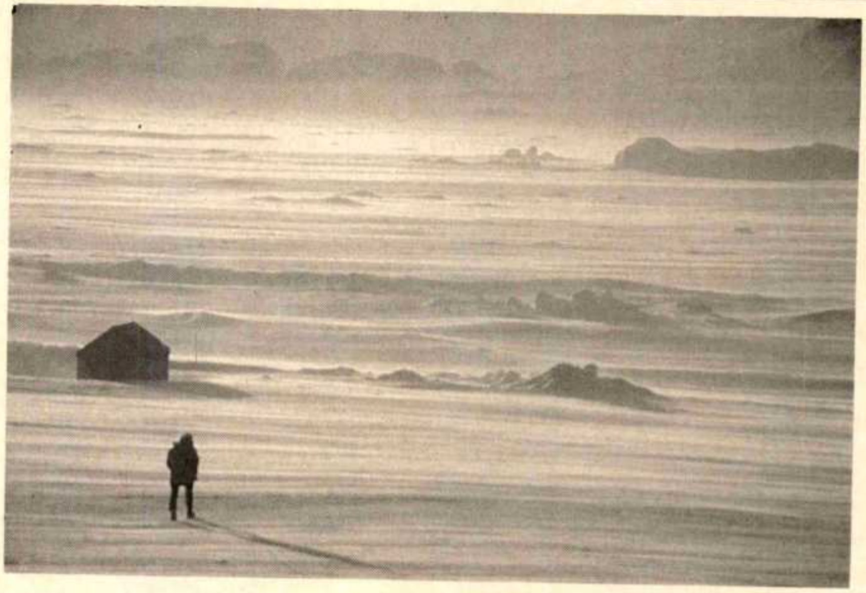
Antarctic Science. Editors in chief David W. H. Walton (life sciences and oceanography), Michael J. Rycroft (atmospheric sciences) and Michael R. A. Thomson (Earth sciences and glaciology). *Blackwell Scientific. 4/yr. North America $132.50 (institutional), $75 (personal); UK £72.50 (institutional), £35 (personal); elsewhere £72.50 (institutional), £42 (personal).*

THE onset of large-scale research programmes in Antarctica during the International Geophysical Year of 1957–58, followed by the creation of the Antarctic Treaty in 1959 (ratified in 1961), and the subsequent continuation of research by many nations, produced a flood of published literature about this unique continent. Much of this literature was dispersed in traditional scientific journals and other serials, and proliferated with the addition of other countries to the original 12 treaty signatories. Control of this literature began in the United States in the early 1960s with the compilation of *Antarctic Bibliography*, a continuing series of volumes that includes annotated citations of all publications about Antarctica. Now we have an all-encompassing journal, *Antarctic Science*, devoted to the publication of research results in three broad disciplines — life sciences and oceanography, Earth sciences and glaciology, and atmospheric sciences. The editors of the journal, the first issue of which was published in March 1989, are three distinguished researchers at the British Antarctic Survey in Cambridge, and are provided with guidance for content by an editorial advisory board of 19 people from 12 countries.

The geographical coverage includes Antarctica, the SubAntarctic (excluding the Falkland Islands, Tristan da Cunha and the New Zealand Shelf Islands), and the Southern Ocean. This generally conforms with the boundaries of the Antarctic Treaty coverage. In addition to review and scientific papers, *Antarctic Science* publishes guest editorials, short notes, meeting reports, a noticeboard, book reviews and special issues. Articles on logistic operations, politics or history are not accepted. Volume 1 contains 37 articles (including reviews) and 3 short notes; the last issue of volume 1 includes a helpful index.

The British Antarctic Survey has taken the lead in starting a much-needed journal of Antarctic science that provides refereed, quality articles by investigators from many countries. This is a considerable challenge, inasmuch as the readership might conceivably consist only of authors' colleagues who are also involved in Antarctic research. Furthermore, there is always the prospect of losing subscribers because of the interdisciplinary content, which might include subjects outside the interests of some specialists. However, Antarctica has passed from the early research of the International Geophysical Year of a general or reconnaissance nature into matters of global consequence, involving depletion of stratospheric ozone, ice-core studies to detect changes in atmospheric chemistry, oceanic and atmospheric warming and possible melting of the Antarctic ice sheet, and investigations into the major carbon sink of the Southern Ocean. This is the strength of the journal, which I believe makes it necessary reading for a wide audience.

Finally, the quality of the scientific content of *Antarctic Science* is equalled by the quality of its production, as confirmed by the fact that the journal received an award in a competition with 140 entries for typographical excellence in journal and serial publishing. ☐



S[illegible] Fraser/Science Photo Library

A drift blowing across sea ice at the British Antarctic Survey base of Rothera, Adelaide Island. The small hut is a pump house for a desalination plant.

John Splettstoesser is at 1 Jameson Point Road, Rockland, Maine 04841, USA.

Flat Earthers

Peter J. Smith

Global and Planetary Change. A Daughter Journal of Palaeogeography, Palaeoclimatology, Palaeoecology. Editors Eric Barron, Sierd Cloetingh, Ann Henderson-Sellers, Diana Liverman, Michael Meybeck, Berrien Moore and Paolo Pirazzoli. *Elsevier. 4/yr. North America $135; elsewhere Dfl. 277.*

THERE has been much talk of late among Earth scientists of global change, global systems and global interactions. NASA calls it Earth System Science, the aim of which is "to obtain a scientific understanding of the entire Earth System on a global scale by describing how its component parts and their interactions have evolved, how they function, and how they may be expected to continue to evolve on all time scales". The International Council of Scientific Unions (ICSU) calls its version the International Geosphere–Biosphere Programme (IGBP), which has almost identical aims. But these are merely the highest strata of the global infrastructure, capping a vast new subculture of committees, programmes, departments and publications.

The US Committee on Earth Sciences is defining a Global Change Research Program; the National Academy of Sciences has set up a Committee on Global Change; the National Oceanographic and Atmospheric Administration has its Panel on Climate and Global Change; and the IGBP has spawned a Scientific Steering Committee and innumerable "coordinating panels" and "working groups". Meanwhile, Rice University in Texas now has an Earth Systems Institute, while Pennsylvania State University has formed an Earth System Science Center. In the NCAR Advanced Study Program, the National Center for Atmospheric Research and seven other institutions are producing teaching modules on global change. ICSU is publishing a regular *Global Change Newsletter*, and the Office for Interdisciplinary Earth Studies in Colorado issues the somewhat more glossy *Earthquest*. And, of course, where scientists boldly go, journal publishers are never far behind. The American Geophysical Union produces *Global Biogeochemical Cycles*, and now Elsevier is publishing *Global and Planetary Change* (*GAPC*).

What can it all mean? Not a lot thus far, and certainly much less than the hype suggests. For one thing, there is little new here in principle. Earth scientists have always been interested in long-term global change — witness studies on worldwide changes in sea level, the growth of continents, compositional variations in the atmosphere, the rate of change of radioactive heat flow with time, ocean–continent interactions, global tectonics, and much else besides.

Is it, then, simply some semantic reformulation designed to attract more financial support in hard times? Partly, perhaps even largely, but not entirely. There is little doubt that scientists' recent ability to link genuine Earth science research with dubious human influences and prophecies of doom (global cooling was the flavour of the 1970s, global warming that of the 1980s) has done wonders in prising open government coffers. On the other hand, through increased knowledge and advancing techniques there should, in theory at least, come a time when it should be possible to make some interesting, and perhaps even important, cross-disciplinary global connections.

That this point has barely been reached, if at all, is well illustrated by the first five issues of *GAPC*, the general aim of which is "to achieve an interdisciplinary view of the causes, processes and limits of variability in planetary change". The range of topics among the 30 or so pages is huge; to give some idea of the diversity it is perhaps sufficient to mention just solar evolution, solar and galactic influences on the Earth, the growth of the Earth's crust, long-term stability of the climate, the hydrological cycle, North American cloud variations, global sea-level changes, the quality of rivers, changes in global carbon, soil contamination in urban areas, mass extinctions, plant evolution and palaeoenvironmental modelling.

Yet interesting and of high quality as many of these papers are, the whole exercise rather misses the mark. For although the journal is conspicuously interdisciplinary, hardly any of the individual papers are multidisciplinary in anything but a trivial sense. Earth scientists write on Earth sciences, chemists on chemistry, biologists on biology, and so on; few, if any, connections are made. The international team of editors (which, incidentally, includes no Briton) might well argue that exposing work in one discipline to scientists in others is worthwhile in itself on the principle that it will increase the chances of cross-fertilization with benefit in the longer term, but I remain sceptical. Most people just don't have the time.

On the evidence of *GAPC*, Earth system science has not proceeded beyond the "component parts", and to suggest otherwise is merely a marketing ploy. As an interdisciplinary review medium, the journal itself is not without its usefulness, but as an advert for the brave new world of global interacting systems it falls rather flat. □

Peter J. Smith is editor of Geology Today *and is in the Department of Earth Sciences, Open University, Milton Keynes MK7 6AA, UK.*

Also submitted for review

THE following is a list of the journals that were eligible for review but that for one reason or another are not covered in the preceding pages. The list does not include journals sent to us that had not published enough titles to be considered.

Annals of Clinical Psychiatry. Official Journal of the American Academy of Clinical Psychiatrists (Elsevier).
Applied and Theoretical Electrophoresis. The Official Journal of the International Electrophoresis Society (Macmillan).
Applied Mathematics Letters (Pergamon).
Astronomy and Astrophysics Review (Springer International).
Discovery and Innovation (Sponsored by the African Academy of Sciences and the Third World Academy of Sciences; published by Academy Science Publishers, PO Box 14798, Nairobi).
Ecological Economics. The Journal of the International Society for Ecological Economics (Elsevier).
Ecological Psychology. A publication of the International Society for Ecological Psychology. (Erlbaum).
European Journal of Mineralogy (*Deutsche Mineralogische Gesellschaft, Società Italiana di Mineralogia e Petrologia* and *Société Française de Minéralogie et de Cristallographie*, in cooperation with the European Mineralogical Union).
Frontiers of Medical and Biological Engineering (VSP).
Games and Economic Behaviour (Academic).
International Environmental Affairs. A Journal for Research and Policy (University Press of New England).
Journal of Applied Phychology (Kluwer).
Journal of Cryptology. (Springer).
Journal of JASTRO. The Official Journal of the Japanese Society for Therapeutic Radiology and Oncology (Pergamon).
Journal of Molecular Endocrinology (Society for Endocrinology).
Journal of Population Economics (Springer International).
Journal of Substance Abuse (Ablex).
Laboratory Robotics and Automation (VCH).
MCSS. Mathematics of Control, Signals, and Systems (Springer International).
Military Psychology. The Official Journal of the Division of Military Psychology American Psychological Association (Erlbaum).
Schizophrenia Research (Elsevier).
Seminars in the Neurosciences (Saunders).
Trends in Endocrinology and Metabolism (Elsevier).

■ *Nature*'s next review supplement will be Autumn Books, which will appear in the 22 November issue.

GUIDE TO AUTHORS

Please follow these guidelines so that your manuscript may be handled expeditiously.

Nature is an international journal covering all the sciences. Contributors should therefore bear in mind those readers for whom English is a second language and those who work in other fields. Please write clearly and simply, avoiding unnecessary technical terminology. *Nature*'s staff will edit manuscripts to those ends if necessary. Contributors should check their proofs carefully.

Because of the competition for space, many of the papers submitted for publication cannot be accepted. For this reason, and because brevity is a great assistance to readers, papers should be as brief as is consistent with intelligibility. Please note that one printed page of *Nature*, without diagrams or other interruptions of the text, has fewer than 1,300 words.

CATEGORIES OF PAPER

Review Articles survey recent developments in a field. Most are commissioned, but suggestions are welcome in the form of a one-page synopsis addressed to the Reviews Coordinator. Length is negotiable in advance but must not exceed six pages of *Nature*.

Articles are research reports whose conclusions are of general interest and which are sufficiently rounded to be a substantial advance in understanding. They should not have more than 3,000 words of text or more than six display items (figures and tables) and should not occupy more than five pages of *Nature*.

Articles should be accompanied by a heading of 50 – 80 words written to advertise their contents in general terms, to which editors will pay particular attention. A heading (printed in italic type) is not an abstract and should not usually contain numbers or measurements. The study should be introduced in more detail in the first two or three paragraphs, which should also briefly summarize its results and implications.

Articles may contain a few subheadings of two or three words. The meaning of the text should not depend on the subheadings, whose function is to break up the text and to point to what follows. There should be fewer than 50 references.

Letters to Nature are short reports of outstanding novel findings whose implications are general and important enough to be of interest to those outside the field. Letters should not have more than 1,000 words of text or more than four display items and should not occupy more than two pages of *Nature*. The first paragraph should describe, in not more than 150 words, the origins and chief conclusions of the study. Letters should not have subheadings or more than 30 references.

Commentary articles deal with issues in, or arising from, research that are also of interest to readers outside research. Some are commissioned, most are unsolicited. They are normally between one and four pages of *Nature* in length.

News and Views articles are intended to inform non-specialist readers about a recently published advance. Suggestions should be made to the News and Views Editor. Illustrations are welcome. Proposals for meeting reports should be agreed in advance.

Scientific Correspondence is for the discussion of scientific matters, including contributions published in *Nature*. Priority is given to contributions of less than 500 words and five references. Figures are welcome.

GENERAL

All contributions submitted for publication in *Nature* should conform with these rules.

Manuscripts should be typed, double-spaced, with a good-quality printer, on one side of the paper only. Four copies are required, each accompanied by lettered artwork. Four copies of half-tones should be included. Reference lists, figure legends and so on should be on separate sheets, all of which should be double-spaced and numbered. Copies of relevant manuscripts in press or submitted for publication elsewhere should be included, clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their reference numbers.

Titles should say what the paper is about with the minimum of technical terminology and in fewer than 80 characters. Authors should avoid active verbs, numerical values, abbreviations and punctuation and should include one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name, figure number and, when known, manuscript reference number. Original artwork should be unlettered. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are permitted only if the parts are closely related, either experimentally or logically. Suggestions for cover illustrations, with captions, are welcome. Original artwork (and one copy of the manuscript) will be returned when a manuscript cannot be published.

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References should be numbered sequentially as they appear in the text, followed by those in tables and finally those in the figure legends. Reference numbers apply only to papers published or in the press, and a different number should be given to each paper cited. All other forms of reference (including unrefereed abstracts) should be included in the text as personal communication, manuscript in preparation or preprint (with number and institution where appropriate). Text should not be included in the reference list. References should be abbreviated according to the *World List of Scientific Periodicals* (fourth edition, Butterworth, London, 1963 – 65). The first and last page numbers should be cited. Reference to books should clearly indicate the publisher and the date and place of publication.

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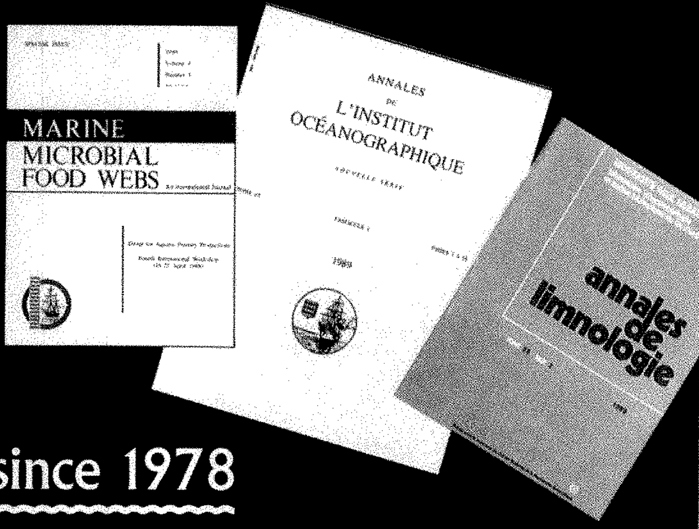
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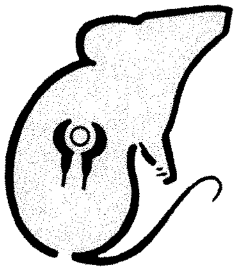

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César Milstein (MRC Cambridge)
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Applicants must present evidence of one publication in research involving solids NMR. Resumes to: Mrs Jimmie Gaston, ALC Specialist, Job Service, 505 Washington, St Louis, Missouri 63101. Refer to Job Order # 413990. (NW5905)A

FACULTY POSITION(S)

MCGILL UNIVERSITY

Department of Pharmacology & Therapeutics

The Department of Pharmacology & Therapeutics is undergoing a phase of growth and expansion, having recently incorporated three members of academic staff with interest in molecular pharmacology. It is now actively searching for suitable candidate(s) for one to two newly created academic tenure track position(s) in the field of Molecular Aspects of Peptide Pharmacology (expertise in peptide isolation, characterization and synthesis is desirable). Preferably, the candidate(s) should have research interests in drug biotransformation, membrane transport and/or chemotherapy. However, the most important consideration is proven excellence in the candidate's (s') field of science. The level of the tenure track position(s) will be commensurate with the qualifications and experience of the selected candidate(s). Salaries for the position(s) start at $37,125, $43,265 and $53,621 respectively for the three levels (Assistant Professor, Associate Professor and Full Professor). The Department is seeking outstanding individual(s) who possess the ability to organize and develop an independent research group.

The McGill Department of Pharmacology & Therapeutics has a broad based group of investigators in neuropharmacology and is very active in the areas of cardiovascular, receptor, developmental, bronchopulmonary and reproductive pharmacology, as well as coxicology. It has a vigorous graduate program which attracts well qualified candidates with great potential. Specialized facilities available include E/M, electronics, photography and advanced image analysis. Financial assistance to establish laboratories and purchase equipment, as well as statutory relocation assistance, will be provided.

Applications, which must include a curriculum vitae, the names and addresses of three referees and a brief outline of proposed research, should be sent to: **Dr A Claudio Cuello, Chairman, Department of Pharmacology & Therapeutics, McGill University, 3655 Drummond Street, Room 1325, Montreal, Quebec, H3C 1Y6, Canada by 15th January, 1991, as the Department hopes to fill this position for the 1991-92 academic year.** (NW5906)A

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MOLECULAR BIOLOGIST

Infectious salmon anaemia (ISA) is a contagious disease which occurs in Norwegian fish farms. It is most probably a virus infection, but so far it has not been possible to isolate the etiologic agent. A multidisciplinary team is investigating ISA by cell culture techniques and by various biochemical and immunological methods. Some studies have already been carried out on the pathological and haematological aspects of the disease.

A molecular biologist is required to join this team. The successful applicant will be responsible for the establishment and application of DNA cloning methods to the isolation and characterization of the causative ISA agent. The DNA work will be carried out at the Department of Biochemistry, Norwegian College of Veterinary Medicine, which houses several research groups in molecular biology and where the necessary laboratory facilities are at hand. The post is funded initially for two years by the Norwegian Council for Fisheries Research.

Qualifications: PhD or an equal degree in a molecular science, relevant experience in DNA methodology and ability to work independently.

Salary: NOK 241,593 (approximately USD 40,000) per annum.

Application, including curriculum vitae, list of publications, information on professional experience and name and addresses of two referees should be sent to National Veterinary Institute, Box 8156 Dep, 0033 Oslo 1, Norway, not later than 5th November, 1990.

For further information Johan Krogsnud, Head of virology section, can be contacted. Tel: (47 2)96 46 60, Fax: (47 2) 46 00 34.

(W7821)A

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Application forms, to be returned by Friday 26th October 1990, and further details are available from the Personnel Department, Polytechnic South West, Drake Circus, Plymouth PL4 8AA, or by telephoning (0752) 232167.

(4835)A

Head of Bioanalytical Unit

Clinical Pharmacology Research Institute Neuss, Germany

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As Head of the Institute's Bioanalytical Unit, you will be involved in the establishment and application of analytical methods for the determination of drug levels in human biological fluids, and the pharmacokinetic interpretation of the data. The Unit comprises some 20 scientists and technicians and is equipped with modern automated GC and HPLC equipment. A new computerised data capture system will be introduced shortly. Immunoassay methods are also used, where appropriate, and some facilities are available for studies of drugs labelled with stable isotopes.

You should have a proven record of achievement in clinical pharmacokinetics, including drug assay, and management experience with a sizeable group working in this field. A good command of English (both written and verbal) is essential and fluency in German, an advantage. You will also maintain regular liaison with similar bioanalytical groups in the UK and United States, and be responsible to the Institute Head with a functional reporting line to the UK Manager of the Drug Metabolism and Pharmacokinetics Department.

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Please send a full cv to the Personnel Director, Clinical Pharmacology Research Institute, SmithKline Beecham Pharmaceuticals, Neuss, Germany.

SB

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Send resume to Dr Barath, 8700 Beverly Blvd, LA, CA 90048.

(NW5900)A

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Applications (12 copies) should be submitted to the Dean, Charing Cross & Westminster Medical School, The Reynolds Building, St. Dunstan's Road, London W6 8RP, from whom a job description should first be obtained. Closing date: 30th November, 1990. (4817)A

UNIVERSITY OF
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Chair of Biochemistry

Applications are invited for the Chair of Biochemistry which has recently become vacant on the retirement of Professor K. Burton, FRS.

The person appointed will be expected to provide academic leadership in research and teaching within the Department of Biochemistry and Genetics. The Department is in the Faculty of Science, is a member of the Cross-Faculty School of Biomedical and Biomolecular Sciences and is located in the New Medical School. This offers opportunities for collaboration within a broad area of research.

Salary will be at an appropriate point on the Professorial salary scale.

Further particulars may be obtained from the Deputy Registrar, the University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU with whom applications, giving the names of three referees, should be lodged not later than 30th November, 1990.

(4822)A

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A Senior Executive Service Vacancy

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Division of Cancer Treatment

Location: Rockville, Maryland

The Associate Director, Developmental Therapeutics Program (DTP), will be responsible for the planning, direction and implementation of a broad national and international program of drug discovery and development designed specifically to maintain a continuous flow of therapeutic agents and materials from laboratory clinics for the treatment of cancer and AIDS. The DTP is composed of three distinct segments with an annual funding level of approximately $117 million. The segments consist of a large extramural contract-based drug-development program (approximately $50 million), a grant-supported Biochemistry and Pharmacology program (approximately $60 million), and an intramural, laboratory oriented research program (approximately $7.5 million) exclusive of salaries and related fringe benefits. The DTP staff consists of approximately 275 scientific and supporting personnel.

This is a Civil Service position in the Senior Executive Service (SES) with an annual salary compensation between ES-1 through ES-4 (currently $71,200 to $79,200). In addition, physicians may be eligible for a Physician's Comparability Allowance up to $20,000 per year. (Applicants alternatively may be eligible for appointment in the Commissioned Corps of the U.S. Public Health Service.) The individual selected, if not presently in SES, must serve a one-year probationary period.

Applicants must meet the minimum educational requirements for at least one of the following: Medical Officer, GS-602; Chemist, GS-1320; Microbiologist, GS-403; Pharmacologist, GS-405; Biologist GS-401; or Health Science Administrator, GS-601; as defined by the U.S. Office of Personnel Management Handbook X-118 (Qualification Standards for Positions under the General Schedule), which is available in Federal Personnel Offices. Applicants must also meet established mandatory professional/technical and managerial/executive requirements. Applicants will be further evaluated on the degree to which they possess additional desirable qualifications in the areas of professional/technical qualifications, managerial/executive qualifications, scientific reputation/peer recognition, training and education, and honors and awards. A copy of the requirements may be obtained by contacting Ms. Sue Hickman in the Personnel Management Branch, NCI, at (301) 496-8182.

Applications for Federal Employment (SF-171) are to be sent to the above individual at:

The National Cancer Institute,
Personnel Management Branch
9000 Rockville Pike
Building 31, Room 3A16,
Bethesda, Maryland 20892.

NATIONAL INSTITUTES OF HEALTH

A current curriculum vitae and bibliography must accompany all applications for entrance into the SES. Public Health Service Commissioned Officers wishing to enter the SES must follow the above procedures. Officers interested in performing the duties of the position *within the Commissioned Corps* may submit a resume to the above address.

Applications must be received by October 26, 1990.

Selection for this position will be based solely on merit, with no discrimination for non-merit reasons such as race, color, religion, sex, national origin, politics, marital status, physical or mental handicap, age, or membership or non-membership in an employee organization.

(NW5819)A

آغا خان یونیورسٹی

THE AGA KHAN UNIVERSITY
Faculty of Health Sciences
Medical College

The Aga Khan University is now in its seventh year of operation. The affiliated Aga Khan University Hospital will have 654 beds when fully operational. The University and the University Hospital are autonomous, privately funded and philanthropic institutions respectively committed to the provision of effective education and health services relevant to Pakistan and the region.

Applications are invited from highly committed professionals for the positions in our **Anatomy** and **Physiology** Departments.

ASSISTANT PROFESSOR/ASSOCIATE PROFESSOR/PROFESSOR DEPARTMENT OF ANATOMY

Anatomy department has wide ranging teaching and research commitments. The subject is taught as a part of well integrated curriculum for Basic Health Sciences. The course runs over five terms of 12 weeks each and consists of cell biology, gross and microscopic anatomy, neuroanatomy and embryology. Present annual intake is 65 students, and English is the medium of instruction.

Candidates applying for these positions are required to have postgraduate degree e.g. Ph.D. in Anatomy and must have five to ten years of subsequent teaching experience in a recognised institution.

ASSISTANT PROFESSOR DEPARTMENT OF PHYSIOLOGY

Candidates applying for this position must have a Ph.D. or an equivalent qualification with 2 years of postgraduate teaching experience in a Medical College and of research relevant to medical sciences. Our undergraduate curriculum in Medicine is intended towards integration of Basic Health Sciences.

Salary and benefits offered will be commensurate with qualifications, experience and level of responsibility. Medically qualified candidates intending to return to Pakistan or candidates intending to pursue a career in Pakistan will be given preference. If you are seeking professional growth and an excellent work environment, please send your detailed resumé, bibliography and names of at least three referees familiar with your recent work history to the **Personnel Director**, The Aga Khan University, P.O.Box 3500, Stadium Road, Karachi-74800, Pakistan.

(W7811)A

FACULTY POSITIONS
Section of Genetics
DUKE UNIVERSITY MEDICAL CENTER

A newly created Section of Genetics at the Duke University Medical Center invites applications for several tenure-track, junior level faculty positions. Although individuals utilizing molecular genetic approaches to questions in most any important area of biology will be considered, we are particularly interested in those applying to genetic approaches to the study of development.

Applicants should forward a curriculum vitae, a statement of research plans and the names of three references to: **Dr Joseph R Nevins, Head, Section of Genetics, Duke University Medical Center, PO Box 3054, Durham, NC 27710.**

Duke University is an equal opportunity/affirmative action employer.

(NW5918)A

UNIVERSITY OF MANCHESTER
SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF CELL & STRUCTURAL BIOLOGY

CHAIR IN PLANT MOLECULAR BIOLOGY

The purpose of this new appointment is to develop an active research group in plant molecular biology in the School of Biological Sciences to complement the other activities of the School and to provide leadership for the Plant Science and Cytogenetics Research Group within the Department. The person appointed will be responsible for courses in higher plant biology which are offered to a range of Honours Schools, including Plant Science and Applied Plant Science. Facilities and opportunities for development are excellent.

For informal enquiries, requests to visit the Department etc, contact Professor Mark WJ Ferguson (Tel: 061 275 6775, Fax: 061 275 6776.)

Applications, consisting of a curriculum vitae, the names and addresses of three persons from whom references may be sought, and a research proposal, should be sent to the Registrar, The University, Manchester M13 9PL, from whom further particulars may be obtained (Tel: 061 275 2028, Fax: 061 273 5306). Closing date: 30th November, 1990. Please quote Ref No 303/90. The University is an Equal Opportunity Employer. (4803)A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF EARTH SCIENCES

MOBIL LECTURESHIP IN TECTONO-SEDIMENTATION AND SEISMIC STRATIGRAPHY

Applications are invited for the above post available from 1st January, 1991 for a period of 5 years.

The Department of Earth Sciences seeks a young scientist with proven ability to research and develop further the understanding of sedimentation and active tectonics in conjunction with Dr Mike Leeder's research group. Candidates should have experience of both active and ancient extensional sedimentary basins and of seismic stratigraphic investigations. An ability to work with and to liaise between academia and industry is essential.

Salary on the scale for Lecturers Grade A (£12,086-£16,755) according to qualifications and experience.

Details of the present staffing, facilities and ongoing sedimentary research can be obtained from the Departmental Administrator (tel (0632) 335254; fax (0532) 335259).

Informal enquiries should be made to Dr Mike Leeder (tel (0532) 335204).

Application forms and further particulars may be obtained from and completed applications forwarded to the Registrar, the University, Leeds LS2 9JT, (tel (0532) 333969 — direct line), quoting ref no 49/52. Closing date for applications: 15th November, 1990.

The University is an equal opportunity employer. (4823)A

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY
P.O. BOX 30772, NAIROBI, KENYA

POSITIONS ANNOUNCEMENT

Applications are invited from suitably qualified candidates for the following positions tenable at the ICIPE Headquarters, Nairobi and Mbita Point Field Station, South Nyanza.

A. LIVESTOCK TICKS RESEARCH PROGRAMME, DUDUVILLE, NAIROBI

PRINCIPAL RESEARCH SCIENTIST
REF: SCI/90/19

The ICIPE's Livestock Ticks Research Programme (LTRP) conducts research into the control of livestock ticks through integrated pest management principles. The main aim is to establish tick control methods which will be sustainable, environmentally safe and cost-effective. Currently, the focus of research is on African ticks. The principal research scientist will be based at the ICIPE Headquarters at Duduville, Nairobi.

Applicants must have a first degree in veterinary medicine, zoology or entomology and a Ph.D. with a minimum of ten years postdoctoral research experience. In addition, they should have a record of outstanding contribution to the advancement of insect science or immunology or population ecology or epidemiology, characterised by qualitative and quantitative scientific publications in refereed journals, presentations at scientific gatherings, distinguished honours and awards, and at least five years of research management experience at a senior level.

The successful candidate will play a leading role in the research and development of integrated tick management packages for tick control in the tropics, with emphasis on application in resource-poor farming communities. He/she will also direct field operations in the process of validation of the tick management packages and test their sustainability. In addition, the successful candidate will be involved in training, specifically to enhance high-level manpower in tick research in tropical developing countries, particularly Africa.

B. BIOLOGICAL CONTROL SUB-PROGRAMME, MBITA POINT FIELD STATION

RESEARCH SCIENTIST
REF: SCI/90/20

This position is tenable at ICIPE's main field station, located at Mbita Point in western Kenya on the shores of Lake Victoria and about 500 km from Nairobi. The field station has residential facilities, including an international primary school, institutional clinic and a guest centre.

Candidates must have a Ph.D. in entomology and at least two years of postdoctoral research experience, as evidenced by refereed publications, in the field of biological control of insect pests. Preference will be given to those who have experience in the use of *Bacillus thuringiensis* for the control of crop pests, especially in the tropics.

The successful candidate will conduct and supervise research on evaluation of *B. thuringiensis* for utilisation in integrated pest management programmes. The research will be carried out in collaboration with a team of scientists under the overall supervision of the programme leader of the Crop Pests Research Programme.

The remuneration package attached to the above-mentioned positions will be commensurate with the qualifications and experience of each appointee.

Applications giving detailed information on education, professional qualifications, experience, marital status, age, present employment (including salary), names and addresses of four referees, photocopies of relevant certificates (which will not be returned), and reprints of key publications, should be addressed to the undersigned. Please quote the advertisement reference number on the envelope.

The Manager for Administration and Information
The International Centre of Insect Physiology and Ecology
(ICIPE)
P.O. Box 30772
NAIROBI
Kenya

Closing date: 11th November, 1990 (W7824)A

HEAD OF DRUG-DISCOVERY RESEARCH

(Medicinal Chemistry & Pharmacology)

ucb

PHARMA RESEARCH CENTER

**Brussels
Belgium**

UCB-Pharma is a pharmaceutical company within the UCB-Group, engaged in long-term research with the aim of developing effective agents for the treatment of disorders of the CNS, the Cardiovascular System and the Immune System.

Our Research Center is looking for a highly skilled, motivated and creative scientist to head its Drug-Discovery Research Unit. This Unit which comprises a staff of 65 includes medicinal chemistry (drug design, chemical synthesis and analytical chemistry) and general (preclinical) pharmacology. The position requires a Ph. D. in medicinal chemistry with expertise in molecular modeling-drug design as well as some experience in pharmacology.

The successful applicant will have an established reputation in research and must demonstrate management skills and abilities to coordinate research activities of highly qualified research teams.

The head of the Drug Discovery unit will report directly to the Director of Pharmaceutical R&D.

We offer a stimulating working environment, with excellent opportunities for personal development.

For further information please contact Dr E. WÜLFERT, Director of R&D, phone 32-2-385.00.10.

Applicants should apply to Mr J. LEYTENS, UCB, Human Resources Management, avenue Louise 326, box 7, B-1050 Brussels.

(W7823)A

MOGAM Biotechnology Research Institute, Korea

SENIOR RESEARCH SCIENTIST POSITIONS

Applications are invited from Korean scientists with a few years of postdoctoral experience in the field of Molecular Biology, Molecular and Cellular Immunology, Nucleic Acid and Protein Chemistry, and Molecular Virology. It is expected that applicants will be able to work independently and be able to lead a research team.

MOGAM Biotechnology Research Institute is a non-profit organizaiton established by the Korea Green Cross Corporation in 1984. The institute is located 15 miles south of Seoul and has excellent research facilities for modern research.

Please send applications, including a curriculum vitae and the names and addresses of two referees, to: Dr. H.M. Moon, AOL, Inc., Suite 403, 1055 Parsippany Blvd., Parsippany, NJ. 07054. Tel: 201 402-5950, Fax: 201 402-5949. (NW5823)A

UNIVERSITY OF SOUTHAMPTON

Tenovus Research Laboratory

A post is currently available in the Tenovus Research Laboratory for a

RESEARCH ASSISTANT

to help with culture facilities for the bulk production of monoclonal antibodies using hollow-fibre technology. The main objective of this project is to provide clinical grade material to a team of scientists and clinicians working on the production of novel antibody derivatives for the treatment of human lymphoma. Experience in cell culture technique and immunoassays would be desirable but not essential. The position is funded for three years and carries a salary, according to age and experience, in the range £11,399 to £13,495. For further details concerning the project contact Dr M Glennie at the Tenovus Research Laboratory (0703) 704144.

Applications including full CV and the names and addresses of two referees should be sent to the Personnel Department, University of Southampton, Highfield, Southampton SO9 5NH, by 2nd November, 1990, quoting Reference No M/65/NA.

Working for equal opportunities.

(4824)A

UNIVERSITY OF UPPSALA, SWEDEN

THE MEDICAL FACULTY

Applications are invited for a

PROFESSORSHIP IN MOLECULAR CELL BIOLOGY

Ref nr 8799/90

Subject area for the chair in Molecular Cell Biology:

This is a new chair in molecular cell biology at the faculty of medicine. The successful applicant will have a PhD, perhaps in combination with an MD and should be active in international front-line research in molecular cell biology of higher eukaryotes of relevance for human disease. He/she will participate in undergraduate teaching of medical students and training of PhD students. Excellent laboratory facilities are available and there are extensive opportunities to collaborate with other research groups at the Biomedical Center of Uppsala which is one of the largest biomedical research facilities in Scandinavia.

The application should be directed to **The Government of Sweden under the address: The Registrator, Uppsala University, Box 256, S-751 05 Uppsala, Sweden,** *no later than 8th November, 1990.*

The applicant should submit a **curriculum vitae** (duly signed and witnessed), **a short written account of his/her scientific and pedagogic activities, certificates and such other documents** as to which the applicant wishes to refer, and **a list of scientific publications. All in four copies.** It should be clear from the written account which results and other scientific achievements the applicant considers should be primary observed in the matter.

The applicant should also indicate and send in those 20 publications which he/she considers most adequate in four copies and the rest of the publications referred to in one copy. Copies of publications should be sent no later than 29th November, 1990.

For further information, please contact **Prof Hans R Ulfendahl, Dean of the Medical Faculty. Telephone 46-18-174182 or Telefax 46-18-55 35 41.**

(W7825)A

UNIVERSITY OF UPPSALA, SWEDEN

THE MEDICAL FACULTY

Applications are invited for a

PROFESSORSHIP IN MEDICAL MOLECULAR GENETICS

Ref nr 8800/90

Subject area for the chair in Medical Molecular Genetics:

This is a new chair in medical molecular genetics at the faculty of medicine. The successful applicant will have a PhD, perhaps in combination with an MD and should be active in international front-line research in medical molecular genetics of relevance for human disease. Experience from work with recombinant DNA technology is mandatory. He/she will participate in undergraduate teaching of medical students and training of PhD students. Excellent laboratory facilities are available and there are extensive opportunities to collaborate with other research groups at the Biomedical Center of Uppsala which is one of the largest biomedical research facilities in Scandinavia.

The application should be directed to **The Government of Sweden under the address: The Registrator, Uppsala University, Box 256, S-751 05 Uppsala, Sweden,** *no later than 8th November, 1990.*

The applicant should submit a **curriculum vitae** (duly signed and witnessed), **a short written account of his/her scientific and pedagogic activities, certificates and such other documents** as to which the applicant wishes to refer, and **a list of scientific publications. All in four copies.** It should be clear from the written account which results and other scientific achievements the applicant considers should be primary observed in the matter.

The applicant should also indicate and send in those 20 publications which he/she considers most adequate in four copies and the rest of the publications referred to in one copy. Copies of publications should be sent no later than 29th November, 1990.

For further information, please contact **Prof Hans R Ulfendahl, Dean of the Medical Faculty. Telephone 46-18-174182 or Telefax 46-18-55 35 41.**

(W7826)A

NATIONAL HIGH MAGNETIC FIELD LABORATORY

Award: A consortium of Florida State University, the University of Florida and Los Alamos National Laboratory has recently been funded by the National Science Foundation to establish a new National High Magnetic Field Laboratory (NHMFL). Two facilities will be established: a DC Magnet Facility in Tallahassee and a Pulsed Field Facility in Los Alamos. The State of Florida is committed to provide 54 Faculty lines; 20 lines will be for visiting faculty and 10 will be for the Scientist/Scholar positions. The remaining 24 positions will be tenure track faculty who with the present faculty and staff of the University of Florida, Florida State University and Los Alamos National Laboratory will build the in-house research program for the NHMFL.

Mission: To establish a state-of-the-art high magnetic field facility driven by an internationally competitive in-house research and development program that is responsive to the needs of the user community, i.e. the creation of a facility capable of supporting research at the extremes of parameter space, i.e., pressure, temperature and magnetic field strength.

Director: The director of the NHMFL will be responsible for the development of the DC Magnet and Pulsed Field Facilities as well as the in-house and external scientific programs. He or she will act upon the advice of the External Advisory Board composed of representatives from industry, universities, national laboratories and user community. A well established international reputation in condensed matter research, and administrative experience is required. The appointment will be as Director of the NHMFL and Professor in an appropriate department. *Deadline for application: December 15, 1990.*

Associate Director of the DC Facility: This Associate Director will be responsible for overseeing the design and development of resistive, superconducting and hybrid magnets for a new generation of high field research. He or she is also responsible for overseeing the 20MW DC power supply, the associated cooling system and shop facilities. Extensive experience is required in magnet development and high field applications as well as significant administrative skill. The appointment will be as Associate Director of NHMFL and will have a faculty position in an appropriate department. *Deadline for application: Dec. 15, 1990.*

Associate Director of the Pulsed Field Facility: This Associate Director will be responsible for explosive generation of kT fields and long pulse ≥ 60T fields achieved with the 1430 MVA CPRF generator. He or she is also responsible for overseeing facility development and operations. Experience in pulsed magnet fields, condensed matter physics, as well as administrative skill, strongly preferred. This position will be at Los Alamos National laboratory, with a joint appointment at FSU/UF in an appropriate department, but send material to the address given below. *Deadline for application: Dec. 15, 1990.*

Head Resistive Magnet Development: This Head will direct the development of DC magnetic field capabilities beyond any currently available. He or she will lead a substantial group of scientists, engineers and technicians in resistive magnet development. Applicants should have extensive experience with Bitter, Polyhelix and Hybrid magnet designs. This is a twelve month appointment with a courtesy appointment in an appropriate department. *Deadline for application: Feb. 1, 1991.*

Head Superconducting Magnet and NMR Development: This Head will direct a program in the development of superconducting magnets for high homogeneity NMR magnets, for very high field research magnets, and for Hybrid magnet development. He or she will also be responsible for directing the development of the associated high frequency NMR spectrometers and for leading a substantial group of scientists, engineers and technicians. The applicants should have extensive experience in the design and development of high field superconducting magnets with high homogeneity, and a current knowledge of superconducting magnet materials. This is a twelve month appointment with a courtesy position in an appropriate department. *Deadline for application: Feb. 1, 1991.*

Head of Operations for the DC Facility: This Head will be responsible for directing the general operations of laboratory magnet programs which will include responsibility for the 20 MW DC power supply, the associated cooling system, the machine, electronics and cryogenic shops as well as the operation of high field magnets. Applicants should have considerable administrative skill and have extensive experience with the operation of high field resistive magnets. This is a twelve month appointment with a courtesy position in an appropriate department. *Deadline for application: Feb. 1, 1991.*

Magnet Design Engineers: Two Engineers will be responsible for developing detailed designs for magnet structures, preparing specifications for outside commercial facilities as well as overseeing the fabrication of the magnets designed in-house. Applicants should have a doctoral degree or the highest degree appropriate in the field of specialization with a demonstrated record of academic research achievement, or professional qualifications in the field of specialization above those which would be equivalent to the highest degree. These are twelve month appointments. *Deadline for application: December 15, 1990.*

Magnet Development Engineers: Two Senior Engineers will carry out detailed resistive magnet designs and assist in preparing specifications for subcontracts. Applicants should have a doctoral degree or the highest degree appropriate in the field of specialization with a demonstrated record of academic research achievement, or professional qualifications in the field of specialization above those which would be equivalent to the highest degree. These are twelve month appointments. *Deadline for application: Feb. 1, 1990.*

Magnet Development Scientists: Two Senior Scholar/Scientist faculty positions are available for applicants interested in carrying out research related to the development of the next generation of high field resistive and superconducting magnets. A strong interest in new materials as well as the physics and engineering of high field magnets is expected. These positions require a doctoral degree or the highest degree appropriate in the field of specialization with a demonstrated record of academic research achievement in high magnetic field technology, or professional qualifications in the field of specialization above those equivalent to the highest degree. These are twelve month faculty positions with courtesy appointments in appropriate departments. *Deadline for application: Feb. 1, 1990.*

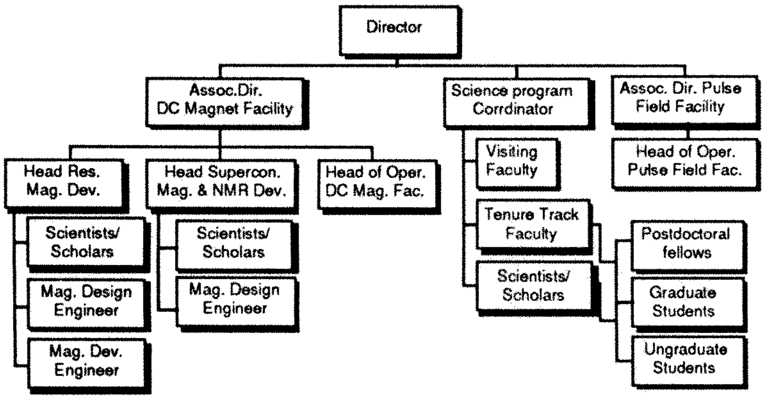
Tenure Track Faculty: Four faculty (experimental or theoretical areas) with academic rank (Professor/Associate Professor or Assistant Professor) in departments are sought who will help build the in-house research program of the NHMFL. The host University will be either the University of Florida or Florida State University and the host department may be Chemistry, Engineering, Physics, or another appropriate department in the sciences. Junior and senior level applicants are encouraged to apply; salary will be commensurate with experience. Among the areas of interest:

- High Temperature Superconductors
- Heavy Fermion Compounds
- Diluted Magnetic Semiconductors
- Quantum Fluids and Solids
- Artificial Structures
- Polymers, Molecular Crystals and Organic Crystals
- Macromolecules/ Liquid Crystals
- Complex Fluids
- High Resolution NMR
- Imaging and in Vivo Spectroscopy
- Microscopic NMR Imaging & Spectroscopy
- Solid State NMR
- One Dimensional Conductors
- High Strength Composites and Insulators
- Layered Structures
- Matrix-Isolated Metal Clusters
- Materials Processing and Devices

These are nine month tenure track appointments. Deadline for application: Feb. 1, 1991.

Applications: Please send applications to:
Dr. Jack Crow, Acting Director, NHMFL
MARTECH, B-159A,
Florida State University,
Tallahassee, Fl 32306.

Include:(1) Letter explicitly stating position for which you are applying and the exact institution and department in which you wish to be affiliated.
(2) CV and publication list
(3) Three letters of reference



Florida State University, University of Florida and Los Alamos National Laboratory are Equal Opportunity/Affirmative Action Employers.

(NW5914)A

CSIRO DIVISION OF WATER RESOURCES
FLINDERS UNIVERSITY OF SOUTH AUSTRALIA
CENTRE FOR GROUNDWATER STUDIES

The Centre for Groundwater Studies is a rapidly growing joint venture of CSIRO, Flinders University, and the SA Government Departments of Mines and Energy, and Engineering and Water Supply (EWS), and is funded as a national centre of concentration of research by the Land and Water Resources Research and Development Corporation.

The Centre's research which focusses on the unsaturated zone, includes:

- land and water salinisation
- groundwater recharge measurement
- evapotranspiration measurement
- contaminant hydrology
- microbiological processes
- hydrochemistry and isotope studies
- palaeo-climate studies

GROUNDWATER MODELLER RESEARCH SCIENTIST/ENGINEER

(Reference No: C2A90)

The Groundwater Modeller post is an applied research position, endowed by the EWS, SA Department of Agriculture (SADA), and the Victorian Salinity Research Program within CSIRO Division of Water Resources.

Responsibilities: Within existing research programs develop and apply groundwater flow and solute transport models for management of groundwater resources in irrigation areas, for assessment of control measures for reducing dryland salinity and for assessing leakages from evaporation basins. Undertake development of specialised models, determine data requirements, calibrate and validate models. Provide training and supervise professionals in the use of groundwater models. Opportunities for lecturing in the School of Earth Sciences at Flinders University and supervision of postgraduate students will also be available.

Qualifications and Experience: PhD in numerical hydrogeology or fluid modelling. Research interests in modelling density-affected flow, unsaturated flow or solute transport are desirable. Demonstrated written and verbal communication skills, and ability to work as part of a team are essential. Experience in field hydrogeology is desirable.

Conditions: A tenured position based at CSIRO Adelaide Laboratory with extended periods at offices of the EWS and SADA. Unlimited use of workstation, mainframe, or PCs. Some out-of-hours work and country travel will be required. The appointee will be a member of the Core Research Group of the Centre, and may be required to undertake occasional consultancies and manage a workstation system.

Level: Research Scientist - Senior Research Scientist
Salary: Aus $34,434 - $53,011

SOIL PHYSICIST - HYDROLOGIST LECTURER

(Reference No: C2B90)

Applications are invited for appointment as Lecturer in Soil Physics/Hydrology in the School of Earth Sciences. The Lectureship is equally supported by Flinders University and by the CSIRO Division of Water Resources.

Responsibilities: The successful applicant will teach course work within the University, assist in the supervision of a growing school of Postgraduate Students and play an active role in the research program of the Centre.

Qualifications and Experience: A PhD or equivalent in soil physics, soil-water-plant interactions or related field is desirable.

Conditions: Located at Flinders University, this position is available initially for a period of three years. The appointee will be a member of the core research group of the Centre.

Level: Lecturer
Salary: Aus $33,163 - $43,096

Further information: on both positions including job specifications and selection criteria are available from Dr Peter Dillon, Centre Director, fax (08) 338 2144, intl fax (618) 338 2144, phone (08) 274 9381, intl phone (08) 618 274 9381.

Applications: giving full details of qualifications and experience, the names and addresses of three referees of whom confidential enquiries may be made, and quoting reference number, should be forwarded, in duplicate to the:

Dean
School of Earth Sciences
Flinders University of South Australia
GPO Box 2100
ADELAIDE SA 5001
by the 30th November 1990

(W7813)A

ABI/SA 90035 *EQUAL EMPLOYMENT OPPORTUNITY IS UNIVERSITY POLICY* 995550

FACULTY POSITION IN CANCER BIOLOGY

The Cancer Biology Section of the Radiation Oncology Center, Mallinckrodt Institute of Radiology, Washington University School of Medicine, St Louis, Missouri has an opening for an individual with training and experience in experimental oncology. Ideally, the candidate should have expertise in the areas of radiation biology and cell profliferation. The opening is available 1st January, 1991. This academic position is tenure-tracked. The salary and rank are negotiable, dependent upon experience and qualifications of the individual. The qualified individual should have the ability to conduct independent research and experience in teaching. The individual will be expected to function as an independent investigator and participate in the training program of the Radiation Oncology Center.

Please send curriculum vitae, 1 or 2 sample reprints and the names, addresses and phone numbers of three references to: Dr Joseph L Roti Roti, Section of Cancer Biology, Washington University School of Medicine, 4511 Forest Park Blvd, St Louis, MO 63108.

(NW5903)A

DEPARTMENT OF PRECLINICAL VETERINARY SCIENCES
R(D)SVS
University of Edinburg
PhD Studentship (MRC)

Applications are invited for a MRC PhD Studentship to be carried out in the above internationally recognised Department and in one of the project areas listed below:

1. Neuropeptides and the Spinal Cord — Professor A W Duggan & Dr S Fleetwood-Walker
2. Neurophysiology and Neuroanatomy of the Spinal Cord — Professor A G Brown
3. Neurophysiological basis of Parkinson's Disease — Dr G Arbuthnott
4. Neuropharmacology of Parasitic Nematodes — Dr R J Martin

Applicants should have a medical, veterinary or good biological science honours degree.

Applications with a CV and the names of two referees should be sent to: Dr R J Martin, Department of Preclinical Veterinary Sciences, R(D)SVS, University of Edinburgh, Summerhall, Edinburgh EH9 1QH. Tel: 031-667 1011 ext 5310.

(4833)A

UNIVERSITY OF PENNSYLVANIA

The Department of Psychology intends to make an appointment at the **Assistant Professor level** in one of the following areas: neuroethology, animal behavior, animal learning, or biopsychology of motivation or learning. Individuals whose research combines more than one of these areas would be especially attractive. We seek candidates with demonstrated excellence in research and teaching. They should submit a vita, reprints and preprints, and a statement of research interests and teaching competence; they should also arrange for 3 letters of recommendation to be sent.

All materials should be sent by 3rd December, 1990 to Search Committee A, Department of Psychology, University of Pennsylvania, 3815 Walnut St, Philadelphia, PA 19104-6196.

The University of Pennsylvania is an equal opportunity/affirmative action employer. (NW5911)A

POSTDOCTORAL POSITIONS

Two grant-funded positions to study: #1 — cell adhesiveness of retinal pigmented epithelium during growth and differentiation; #2 — function of Ca-binding proteins in non-muscle cells. Experience in protein biochemistry and/or molecular biology is highly desirable.

Send CV, research interests and the names of three references to: Dr M Opas, Department of Anatomy, University of Toronto, Med Sci Bldg, Toronto, Ontario, M5S 1A8, Canada.

(NW5907)A

British Bio-technology Limited

Institute of Molecular Medicine, Oxford

British Bio-technology is a research and development company in health sciences. Utilising both molecular biology and medicinal chemistry techniques, the company aims to bring innovative pharmaceutical products to the human health care market in the 1990's and beyond.

Institute of Molecular Medicine, Oxford in collaboration with British Bio-technology Limited, Oxford

POSTDOCTORAL SCIENTIST - MOLECULAR IMMUNOLOGY

Salary range £11,000 - £18,500
Academic Research Staff Grade 1A
Closing date 16th November, 1990

A three-year postdoctoral fellowship is available in the Molecular Immunology Group, Institute of Molecular Medicine, Oxford.

The appointed scientist will be involved in developing prokaryotic and eukaryotic expression systems for HLA class I and class II molecules. This work will be done in collaboration with British Bio-technology Limited where expertise in a wide range of such expression systems is currently available. The protein products obtained from a successful expression system will be utilized to determine structures of bound peptides using nmr. Experience in molecular biology or protein biochemistry is desirable.

Informal enquiries can be made through **Professor Andrew McMichael (tel: 0865 752336) or Dr. John Bell (tel: 0865 752330).** Application forms can be obtained from **Dr. G. Warner, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, U.K.**

British Bio-technology Limited

(4838)A

LIVERPOOL HEALTH AUTHORITY

Department of Clinical Pathology

CLINICAL BIOCHEMISTS — 2 POSTS

Post (1) Grade B salary £15,999-£17,304
Post (2) Grade B salary £12,644-£13,676

The department recently affiliated to the University of Liverpool, is part of a major teaching hospital, covers a comprehensive analytical service and has its own 4-bedded metabolic unit and associated clinics.

You will be expected to have previous clinical laboratory experience and to contribute to the routine and development work of the department. Post (1) — development of interests associated with nutrition and metabolism, cell culture and bio assay or molecular biology associated procedures; post (2) — in the trace and toxic metal section.

Qualifications required are a first or second class honours degree in biochemistry or related subject and preferably a higher degree PhD and/or MCB/MRCPath. Opportunites will be available to study for a professional qualification if required.

Further information is available from Professor A Shenkin or Dr N B Roberts. Tel: 051 706 4232/051 706 4234.

Application forms can be obtained from the Personnel Department, Royal Liverpool Hospital, Prescot Street, Liverpool L7 8XP. Tel: 051 706 2810. Closing date: 26th October, 1990.

An equal opportunities employer. (4805)A

Leicester University

Department of Microbiology

POSTDOCTORAL RESEARCH ASSOCIATE

Molecular Biology of H.I.V.

Applications are invited for a Post-doctoral Research Associate to work on the molecular biology of Human Immunodeficiency virus. This project, funded by the MRC AIDS Directed Programme for three years, will aim to identify molecular determinants which are the basis for HIV cell tropism. Previous experience of molecular biology and/or virology would be an advantage, but not essential. Salary on RA IA scale (£11,399-£18,165 p.a. depending on age and experience).

Applications (as soon as possible) and informal enquiries to: **Dr. Alan Cann, Department of Microbiology, University of Leicester, University Road, Leicester LE1 9HN. Tel: 0533 522951.** (4766)A

Temple University School of Medicine

Positions at all faculty ranks are available in the areas of **receptors, signal transduction, growth factors, developmental biology, immunology** and **molecular immunology.** Candidates are expected to have a strong research background. Facilities are excellent and a variety of shared resources are available. Applicants should submit curriculum vitae, a statement of future research interest and three letters of recommendation to:

Dr. Gerald Litwack, Deputy Director
Fels Institute for Cancer Research
and Molecular Biology
TEMPLE UNIVERSITY
SCHOOL OF MEDICINE
3420 North Broad Street
Philadelphia, PA 19140

An equal opportunity employer.

(NW5898)A

UNIVERSITY

Choose
TEMPLE

The University of Sydney

AUSTRALIA

CHAIR OF BIOLOGY

Reference No. 38/01

Applications and expressions of interest are invited for appointment to a Chair of Biology in the area of animal biology, in the School of Biological Sciences, which will become vacant on the retirement of Professor D T Anderson FRS in December 1991. There are three other Chairs in the School, with current responsibilities in vertebrate biology, plant and cell biology and genetics and molecular biology. The School has responsibilities for teaching in all areas of biology including physiology and ecology of animals, fungi and plants, marine biology, developmental biology, genetics and molecular biology. The Chair may be held in any area of animal biology, but preference may be given to applicants with an interest in teaching and research involving invertebrates.

Further general information is also available from the Association of Commonwealth Universities, 36 Gordon Square, London, WC1H OPF.

The University reserves the right to appoint by invitation and not to proceed with any appointment for financial or other reasons.

Salary: $A67,812 per annum.

Applications (in triplicate for Chairs) quoting reference no., and including curriculum vitae, list of publications and the names, addresses and fax. nos., of three referees, to the Registrar, Staff Office, University of Sydney, NSW, Australia 2006, from whom general information is available by 20 December, 1990.

Equal employment opportunity and no smoking in the workplace are University policy. (W7810)A

61661

Leicester University

Department of Biochemistry
RESEARCH ASSOCIATE

Applications are invited from candidates to join a well-equipped laboratory investigating the dynamics of proteins involved in muscle contraction. Specifically, the characterisation of conformational changes in myosin will be carried out with regard to regulation of ATPase activity and filament assembly (see *J. Molec. Biol.* 1988 203, 173-181; *J. Cell Biol.* 1989, 109, 549-556.) Methods used will include stopped-flow and fluorescence spectroscopy using state of the art commercial and custom-built instruments.

Applicants should have a background in protein/physical biochemistry or muscle physiology. The post is tenable for up to 3 years starting from 1st January 1991 on the RA IA scale (£11,399-£18,165 pa) according to qualifications and experience.

Applications, including a CV and the names of two referees should be sent to **Dr C R Bagshaw, Department of Biochemistry, University of Leicester, Leicester LE1 7RH (tel: 0533 523454)** from whom further details may be obtained.

Closing date for applications: 16th November, 1990.

(4810)A

UNIVERSITY COLLEGE LONDON
Department of Medicine
CELL BIOLOGIST

Applications are invited from an experienced post-doctoral cell biologist for the post of Research Assistant to work as part of an active multidisciplinary group on various aspects of the function of components of the NADPH oxidase electron transport chain of neutrophils and macrophages, with particular attention to the interaction between the oxidase and the cytoskeleton.

The work will involve cell culture and separation, immunohistochemistry, protein purification, antibody production and Western blotting. The appointment is funded by the Wellcome Trust for two years in the first instance, although it is expected that this will be extended for at least a further three years.

Salary will be on the Research Assistant 1A or 2 scales to a maximum of £21,134 pa plus £1,767 London Weighting, depending upon age and experience.

Applications, including full CV and names and addresses of two referees, to Prof A W Segal, Department of Medicine, UCL, Rayne Institute, Unive Street, London WC1E 6JJ, from whom further details can be obtained. Informal enquiries can be made by telephone: 071-380 9725 or Fax: 071-380 9837. *Equal Opportunities Employer.* (4816)A

THE UNIVERSITY OF ADELAIDE — SOUTH AUSTRALIA
invites applications from both women and men for a

LIMITED-TERM LECTURESHIP IN POPULATION AND EVOLUTIONARY GENETICS

available from 1 February 1991 to 31 December 1991 in the **DEPARTMENT OF GENETICS**. The appointee will be responsible for a course of thirteen lectures and associated tutorials in this area given to final year B.Sc. students in Semester I.

This course finishes in mid-July 1991. The appointee will also be responsible to give some related lectures and conduct practicals in earlier years of the course in both semesters. Expressions of interest are sought both from suitable applicants who are available for the whole period and from those who may be interested in a shorter period of employment which would accommodate most of the teaching requirement. Applicants should hold a Ph.D. degree or have equivalent professional training and experience in an appropriate discipline. Further information may be obtained from Dr D. L. Hayman, Acting Head, Department of Genetics. Annual Salary: AUS$33,163 x 7 — AUS$43,096.

APPLICATIONS IN DUPLICATE, quoting reference number 1825, giving personal particulars (including whether candidates hold Australian permanent residency status), résumé and names and addresses of three referees should reach the Director, Personnel Services, University of Adelaide, GPO Box 498, Adelaide, South Australia, 5001, Telex UNIVAD AA 89141, Facsimile (61 8) 223 4820 not later than 12 November 1990.

THE UNIVERSITY OF ADELAIDE IS AN EQUAL OPPORTUNITY EMPLOYER.

(W7816)A 961603

agc

PLANT SCIENTISTS

Molecular Biology, RFLPs and Biochemistry

CAMBRIDGE **COMPETITIVE SALARY**

The Agricultural Genetics Company (AGC), is developing technology for the improvement of crop plants and environmentally safe control of pests and diseases. AGC is currently expanding its in-house Applied Plant Technology Laboratory which is based at Babraham, Cambridge. This new laboratory has state-of-the-art facilities for plant molecular biology, tissue culture and production of transgenic plants. Applications are invited for the following posts:

MOLECULAR BIOLOGY

Senior Research Scientists

Two Molecular Biologists will be responsible for leading AGC's research in the identification and cloning of plant genes. Current projects include research aimed at resistance of plants to insects and viruses, hybrid systems and the enhancement of oil quality in oilseed rape. Applicants should have a PhD and relevant experience.

Research Scientists

Two Molecular Biologists are required to join the teams working on specific projects. Applicants should have experience in gene cloning, a good understanding of plant biochemistry and preferably a PhD.

RFLPs

Project Leader

The RFLP scientist will be responsible for leading AGC's research on the application of RFLPs as an aid to selection in oilseed rape, wheat, barley and peas. This will involve close collaboration with AGC's plant breeding company, Cambridge Plant Breeders Ltd., as well as with AFRC research groups who are developing RFLP probes/maps in these crops. Applicants should have a PhD and preferably experience in plant genetics.

BIOCHEMISTRY

Research Scientist

A biochemist is required to join a team investigating low-temperature sweetening in potatoes. The project will involve protein purification, enzyme assays and metabolic analysis of transgenic plants. This post is based at the AFRC Institute of Arable Crops Research, Rothamsted, Harpenden, Herts., but will involve collaboration with AGC scientists at Cambridge. Applicants should preferably have a PhD.

AGC offers a stimulating working environment together with a competitive salary package, including BUPA and company pension/life assurance. The posts are available immediately and applications should be sent to Dr WDO Hamilton, Applied Plant Technology Laboratory, Babraham, CB2 4AZ. The closing date for applications is November 2 1990.

(4843)A

UNIVERSITY OF CAMBRIDGE
Department of Pharmacology

POSTDOCTORAL POSITIONS

Applications are invited for two postdoctoral research associate positions in the epithelial research group. One project aims to characterise receptors for gastrin-like peptides in epithelial cells. The other will be concerned with the pharmacology of epithelial chloride channels or with the CFTR protein which is abnormal in cystic fibrosis. The posts are available at once, for 3 years with possible extension, and on the RA 1A scale.

Applications, including a CV, plus the names of two referees, should be sent to Prof A W Cuthbert, Department of Pharmacology, Tennis Court Road, Cambridge CB2 1QJ. Previous experience with either receptor binding, electrophysiological techniques or molecular biology would be an advantage. (4837)A

THE UNIVERSITY OF MANCHESTER
Department of Geriatric Medicine

RESEARCH ASSISTANT

Applications are invited for this post in the University Department of Geriatric Medicine, Hope Hospital. The post will be for two years in the first instance and will commence from 1st December, 1990 or as soon as possible thereafter. Candidates should have a good honours degree in biological science, preferably in physiological science. The appointee will join a team based in the Universities of Liverpool and Manchester working on new methods of treating muscle wasting in elderly subjects, using electrotherapy based on the natural firing patterns of motor units. The Specific role of the Research Assistant will be to determine the firing patterns using analytical electromyography. The initial salary will be £11,399 pa rising according to the scale for Research Staff Grade 1B.

All enquiries to Professor Raymond Tallis, University Department of Geriatric Medicine, Hope Hospital, Eccles Old Road, Salford M6 8HD (Tel: 061 787 7164) to whom applications should be sent by 26th October, 1990. The University is an Equal Opportunity Employer.

(4839)A

The University of Waikato
Hamilton, New Zealand

POSTDOCTORAL FELLOWS IN THERMOPHILE RESEARCH UNIT

The University invites applications from suitably qualified microbiologists for two postdoctoral positions in the Thermophile Research Unit.

For the first position candidates should ideally have a sound background in microbial physiology/microbial ecology. The ability and willingness to work well as member of a team from time to time is essential. The position involves some collaborative research with other group members and in meeting requests from commercial organisations for samples and screens. Experience in some of the following areas of research would be desirable: thermophilic bacteria, screening for new organisms and activities, fermenter and continuous culture operation, optimising culture growth or enzyme expression. The appointment will be made at the Junior Lecturer/Lecturer level, with a salary range from NZ$31,200 to NZ$49,088 per annum.

The second position is for a microbial ecologist with an interest in applying nucleic acid probe procedures to the ecology of selected thermophilic bacteria. Some experience in the construction and application of DNA primers would be useful. It is likely that the successful candidate will conduct some of his/her research at the Centre for Gene Technology in Auckland University. This position has a limited tenure of 16 months and will be at a salary of not less than NZ$35,000.

The Thermophile Research Unit is well equipped and partly funded by contract research. It is situated in New Zealand's largest inland city and provides easy access to extensive thermal regions and a variety of other recreational areas.

Enquiries of an academic nature may be made to Professor Hugh Morgan (tel. (64) 71 562889; Fax (64) 71 560 115). Information on the method of application and conditions of appointment can be obtained from the Academic Staff Registrar, University of Waikato, Private Bag 3105, Hamilton, New Zealand (tel. (64) 71 562 889, Fax (64) 71 560135). Applications, quoting reference number A90/90 close on 30th November, 1990.

Places for appointees' children may be available in the creche run by the Campus Creche Society (Inc). The University welcomes applications from suitable people regardless of race, creed, marital status or disability.

(W7820)A

THE STATE UNIVERSITY OF NEW JERSEY
RUTGERS
Campus at Newark

CENTER FOR MOLECULAR & BEHAVIORAL NEUROSCIENCE

CELLULAR/MOLECULAR NEUROSCIENTISTS

with well developed interests in inter- & intracellular signalling mechanisms and/or gene expression/regulation in adult or developing nervous systems. Two of fourteen state-funded, tenure-track faculty positions are available for appointment at the Assistant to Full Professor level with a start date of July 1991. Neuroscientists stimulated by the opportunities offered by the integrative focus and collaborative environment of the Center in the areas of molecular and behavioral research are encouraged to apply. State-of-the-art laboratories and equipment for human, animal, cellular and molecular research, library and conference facilities are available in the new research Center on the Rutgers-Newark campus. The Center is in close proximity to (and with joint appointments possible in) the Institute of Animal Behavior and the Departments of Psychology, Chemistry and Biological Sciences at Rutgers, and the New Jersey Medical School. The Newark campus is close to both New York City and attractive New Jersey countryside living.

Interested neuroscientists should send their CV, names of three references and a brief letter of research goals and philosophy to Dr Ian Creese, Center for Molecular & Behavioral Neuroscience, 195 University Avenue, Newark, NJ07102, USA.

Rutgers is an affirmative action/equal opportunity employer. (NW5910)A

UNIVERSITY OF OXFORD

EXPERIMENTAL PHYSICIST: SPACE RESEARCH

Applications are invited for a permanent position as University Lecturer in Physics, with the possibility of a Tutorial Fellowship at Trinity College. The successful candidate will be an experimentalist with an interest in space instruments and will be expected to do research in the area of Atmospheric, Oceanic and Planetary Physics, but previous experience in these specialities is not essential.

Further particulars of the Lecturership and of the College Fellowship may be obtained from Professor F W Taylor, Department of Atmospheric, Oceanic and Planetary Physics, Clarendon Laboratory, Oxford OX1 3PU, England. Tel: (0865) 272933. (4830)A

PhD IN BIOCHEMISTRY

with experience in lens biochemistry, immunochemistry, cell culture. Independent investigator capable of directing teaching and research.

Send CV to James P McCulley, MD, Ophthalmology Dept, UT Southwestern Medical Center, 5323 Harry Hines, Dallas, TX 75235-9057. Equal Opportunity Employer. (NW5915)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH

DIVISION OF PATHOLOGY

RESEARCH SCIENTIST

A post doctoral or graduate research scientist is required to join a team working on simian immunodeficiency viruses. Our studies are centred on the development of experimental vaccines and analysis of protective immune reponses. Preference will be given to candidates with an interest and experience in cell-mediated immunology. The project funded by the MRC AIDS Directed Programme will involve extensive collaboration with workers in Professor T Lehner's laboratory at Guy's Hospital. Informal enquiries may be made to Dr Martin Cranage or Dr Peter Greenaway on 0980-610391.

The appointment will be made on the Clinical Scientist Scale £9,239 to £15,999 dependent upon age, experience and qualifications.

National Health Service terms and conditions will apply.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Do Salisbury, Wilts SP4 0JG, 0980-610391 ext 2240, to wh completed forms should be returned by 26th October, quoting Post No 1130.

An Equal Opportunities Employer. (4814)A

FACULTY POSITION

The Department of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn, NY, invites applications for a faculty position at any level in an actively growing department committed to expanding its faculty in the general area of molecular, cellular and neurobiology. The position will require teaching in our medical school histology/cell biology, neuroanatomy or gross anatomy course and participation in graduate school core curriculum. Candidate must have an MD or PhD degree with extensive experience and demonstrated ability to conduct an independent research program. Candidates with ongoing programs of excellence in the general areas of mechanisms of gene expression, and regulations of growth factor gene expression, signal transduction mechanisms with expertise in protein biochemistry and DNA/protein interactions are particularly encouraged to apply. Excellent laboratory facilities, start-up funds and infra-structural support and excellent environment for collaboration among basic and clinical investigators are available for modern biological work.

Send curriculum vitae, a statement of research goals, and the names of three references, to: **Dr M A Q Siddiqui, Professor and Chairman, Department of Anatomy and Cell Biology, Box 5.** (NW5901)A

State University of New York
HEALTH SCIENCE CENTER AT BROOKLYN
450 Clarkson Avenue, Brooklyn, N.Y. 11203
EO/AA Employer DMC #C0342

Sloan-Kettering Institute for Cancer Research

Immunology Program

Postdoctoral position available to study molecular and cellular aspects of T cell development. Specific experiments involve studying molecular requirements for positive and negative selection, control of T cell receptor, CD8 and CD4 expression and signaling via these molecules at different stages of intrathymic development. Generation and analysis of transgenic mice using intrathymic injections, fetal thymic organ culture and other molecular and cellular techniques are going to be used to answer these questions. Experience in molecular techniques is an asset. Memorial Sloan-Kettering Cancer Center is an excellent intellectual environment, conducive to productive research.

Applications consisting of a cv, list of publications and at least two letters of recommendation should be submitted to:

Dr. Janko Nikolic-Zugic
Head, T Cell Development Laboratory
Memorial Sloan-Kettering Cancer Institute
1275 York Avenue
New York, NY 10021
(212) 639-2387

SKI is committed to equal opportunity through affirmative action and therefore, members of minority groups are encouraged to apply.

(NW5927)A

POSTDOCTORAL POSITION

Roche Institute of Molecular Biology

Postdoctoral applicants with a background in cell and developmental biology are invited to reply. Research will focus on the establishment and decoding of positional information and gonad development in *Drosophila*. Full funding available.

The Roche Institute is conveniently located in the New York Metropolitan area, 10 miles west of Manhattan. Send curriculum vitae, a description of research interests, and the names of three references to: **Dr. Ken Howard, Roche Institute of Molecular Biology, Department of Cell and Developmental Biology, Nutley, New Jersey 07110.** We are an equal opportunity employer.

Roche Institute of Molecular Biology
ROCHE Hoffmann-La Roche Inc.
(NW5922)A

UNIVERSITY OF UTAH
Department of Biochemistry
TWO TENURE TRACK POSITIONS

are available for autumn 1991 in the area of physical biochemistry. We are looking for strength and originality in the determination of the structures of macromolecules, elucidation of their mechanisms of action, and characterization of reaction energetics. Individuals expert in NMR, optical methods, thermodynamics, kinetics, or x-ray crystallography are particularly encouraged to apply.

Applicants should hold a doctoral degree and have a strong record of original research. Preference will be given to applicants at the level of Assistant Professor, but candidates at more senior ranks will also be considered. Successful candidates will join an interactive and diverse faculty and participate in vital campus-wide graduate programs.

Send curriculum vitae, a statement of research background and interests, and three letters of recommendation to: **Dana Carroll, Faculty Search Committee, Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT 84132.**

Deadline for applications is December 1st, 1990; late applications will be considered if openings are still available. Equal opportunity, affirmative action employer.

(NW5653)A

SCHOOL OF PHARMACY
University of London
TWO LECTURESHIPS IN PHARMACEUTICAL CHEMISTRY

Two Lectureships are vacant in the Department of Pharmaceutical Chemistry, which has a wide range of research programmes in the area of synthetic chemistry, receptor biochemistry, lipid biochemistry, enzymology, metabolism, NMR spectroscopy, mass spectroscopy, peptide and protein chemistry, chemical formulation, electrochemistry and optical spectroscopy, and which teaches students studying for pharmacy and toxicology degrees at Bachelors and Masters levels.

One post is to be filled with a person with interests and experience in medicinal organic chemistry, pharmaceutical analysis, biochemistry or molecular biology.

The second post is a fixed term appointment for one year in the first instance and to be filled by a person with training and interests in mass spectrometry.

Salary for Lecturer Grade A in the range £13,853-£18,522.

Applicants should send two copies of full CV, a research proposal and names of 3 referees to: Mr N J Rampley, Assistant Secretary, 29-39 Brunswick Square, London WC1N X.

(4808)A

CHAIR OF ARCHAEOLOGY

Applications are invited for the newly-established Chair of Archaeology in the School of Archeology, Classics and Oriental Studies.

The University is seeking candidates with an expertise in the methods and theory of archaeology, together with an area or period specialisation.

The salary will be within the range approved for non-clinical professorial salaries, at present not less than £28,252 per annum.

Prospective candidates are invited to contact Professor John Davies, Head of the School of Archaeology, Classics and Oriental Studies, (051-794 2400) for informal enquiries.

Applications, by c.v. with the names of three referees, should be received by the Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained.

Quote ref: RV/824/N

Closing date: 30 November, 1990.

(4836)A

University of LIVERPOOL

AN EQUAL OPPORTUNITY EMPLOYER

THE INSTITUTE OF CANCER RESEARCH
(University of London)
POSTDOCTORAL SCIENTIST
Radiotherapy Research Laboratories, Sutton, Surrey

A Postdoctoral Scientist is required to join a team of scientists who are investigating the radiobiology of human tumours. This is an exciting area of research at the present time, in which there are good prospects for increasing our understanding of the clinical response of cancer to radiation treatment. The work of the Department currently focuses on the nature of differences in radiosensitivity among human tumours, including studies of cell survival, DNA damage, and molecular aspects of repair. We also have an interest in modifers of radiation sensitivity, and prediction of tumour and normal-tissue responses.

The successful candidate will be expected to develop a specialist area of research within this general programme. Experience in cellular or sub-cellular radiobiology would be an advantage.

The post will be for an initial period of 3 years. Salary will be in the range £15,262 pa to £18,522 pa inclusive.

To apply send a curriculum vitae in duplicate, with the names and addresses of two referees, to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference 10.90.S.N.29. (4804)A

SENIOR APPOINTMENT

The **Department of Ecology and Evolutionary Biology at Princeton University** plans to appoint a **Full Professor** with theoretical or experimental interests in ecology, evolution or behavior. Candidates should be willing to take on senior administrative responsibilities.

The current faculty in the Department are: Andrew Dobson, James Gould, Peter Grant, Henry Horn, Steven Hubbell, Martin Kreitman, Carlos Martinez del Rio, Naomi Pierce, Dan Rubenstein, Donald Stratton and Ted Sullivan. The closing date for applications is 30th November, 1990.

Applicants should send a curriculum vitae and arrange for three letters of reference to be sent to **Peter Grant, Search Committee, Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544-1003.**

Princeton University is an equal opportunity/affirmative action employer. (NW5919)A

UNIVERSITY OF LONDON INSTITUTE OF PSYCHIATRY

Departments of Neuropathology/Psychiatry
MOLECULAR CYTOGENETICS RESEARCH MLSO GRADE 1 OR 2

Applications are invited from suitably qualified candidates with the FIMLS or with appropriate experience to join a broad-based team investigating the molecular cytogenetics of mental handicap and cytogenetic disorders. The successful candidate will join a lecturer in providing the Clinical Cytogenetics Service and research facility for the Bethlem Royal and Maudsley Hospitals, which will be based within the Cytogenetics Unit of Guy's Hospital, London Bridge. There will also be opportunity to participate in ongoing research programs in mental handicap and psychiatric disorders, and publication in scientific journals will be encouraged.

Candidates with experience in cytogenetics are particularly invited to apply, but since full training will be provided in the relevant techniques, candidates with experience in related areas who wish to move into cytogenetics are also encouraged to apply.

Starting salary is according to the Whitley Council scale (up to £15,891). Informal enquiries about the post are welcome. Please contact Dr D A Collier at the Institute of Psychiatry on 071 703 5411 ext 3343. Further written details and application forms are available from the **Personnel Office, The Institute of Psychiatry, DeCrespigny Park, Denmark Hill, London SE5 8AF.** The closing date for applications is the 7th November, 1990. Please quote Ref 90/R35.

(4834)A

UNIVERSITY OF MANCHESTER
SCHOOL OF BIOLOGICAL SCIENCES
Department of Biochemistry & Molecular Biology

POST-DOCTORAL RESEARCH ASSOCIATE
Structural Analysis of the Yeast Ty Virus-Like Particle

Applications are invited for this post to join a group working on the structural and molecular organisation of the Ty virus-like particle of yeast. This particle is currently being engineered as a novel polyvalent antigen carrier for vaccine development in a number of areas including AIDS. The appointee will work on the molecular architecture of the Ty particle and hybrid Ty-HIV particles and should ideally have experience of electron microscopy or other biophysical approaches to the study of biological structures. The project is funded by SERC as a cooperative grant between the University and British Bio-Technology Ltd, Oxford.

Salary in range £11,399-£18,165 pa, according to qualifications and experience.

Further details from Professor K Gull, Department of Biochemistry & Molecular Biology, the University, Manchester M13 9PT, to whom applications with a curriculum vitae and names of referees should be sent by October 26th. The University is an equal opportunity employer. (4825)A

PLANT GENETICS
Indiana University, Bloomington

The Department of Biology is initiating a search for an assistant professor of plant genetics. Individuals active in any aspect of plant genetics are encouraged to apply. The successful candidate will be expected to conduct a vigorous, independent research program and contribute to teaching. Applicants should have postdoctoral research experience and demonstrated ability as creative scientists.

Send curriculum vitae and a brief description of research interests, and arrange to have three letters of reference sent to: Plant Genetics Search Committee, Department of Biology, Indiana University, Bloomington, IN 47405.

Applications received before 1st December, 1990, will be assured of consideration.

Indiana University is an Affirmative Action/Equal Opportunity Employer. (NW5904)A

THE UNIVERSITY OF LEEDS
DEPARMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the post of Research Fellow in the Department of Biochemistry and Molecular Biology to undertake investigations involving the chemical synthesis of model mono and oligosaccharides related to the glycosaminoglycan polysaccharides. The compounds will be characterised by X-ray crystallography and NMR spectroscopy to yield stereochemical data which are required for an understanding of how sulphate and carboxyl groups bind cations and influence sugar ring conformations in carbohydrates. The proposed investigations form part of a wider study, the aim of which is to provide more detailed knowledge of the molecular forces and conformational transitions which govern interactions of the molecular recognition type involved in many important biological functions.

A good honours degree and PhD in Chemistry are required and experience with modern synthetic and purification procedures in the carbohydrate field would be an advantage.

The post is available from 1st January, 1991, for a fixed period of up to two years and is supported by SERC.
Salary within the range £11,399-£13,495 on the IA scale for Research and Analogous Staff, according to qualifications and relevant experience.

Informal enquiries may be made to Dr W Mackie (Tel: 0532 333039).

Application forms and further particulars may be obtained from, and completed applications forwarded to, the Registrar, the University, Leeds LS2 9JT (tel (0532) 333966 – direct line), quoting ref no 83/106. Closing date for applications: 22nd November, 1990.

The University is an equal opportunities employer. (4832)A

UNIVERSITY COLLEGE DUBLIN
Centre for Water Resources Research

POST-DOCTORAL RESEARCH FELLOW (Funded)

Required to assist in externally funded research project:

SPATIAL VARIABILITY OF LAND SURFACE PROCESSES

Applicants must have extensive research experience in hydrological modelling and system theory, and an interest in the spatial variability of land surface processes.

The appointment will be for one year at an annual salary of £13,125 inclusive of all deductions.

Applications, including the names and addresses of two referees, should be sent not later than 5th October to:

Mr Aodh Dowley,
Centre for Water Resources Research,
Department of Civil Engineering, University College,
Earlsfort Terrace, Dublin 2.
Tel: (0001) 693244 Ext. 6341.

from whom further particulars may be obtained. (4818)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH
DIVISION OF BIOLOGICS
EUROPEAN COLLECTION OF ANIMAL CELL CULTURES
DATA MANAGER

The European Collection of Animal Cell Cultures is an International Depository Authority for Patent Deposits and has a service and supply function to industry and academic research departments. It is also involved in new initiatives related to the Cell Banking aspects of the Human Genome Mapping Project funded by the MRC and the International Hybridoma Databank Project by the EEC.

A graduate scientist in a suitable discipline (eg Microbiology/Biochemistry/Immunology/Cell Biology) is required for coordinating the Collection's activities in these and other projects. A good knowledge of computing is required to assist scientific staff of the Collection in the work and help maintain existing data and communications on Dell and Apricot XEN-i networks.

The appointment will be made on the Clinical Scientist Scale £17,304 to £18,716 (pay award pending).

National Health Service terms and conditions will apply.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG, Tel: (0980) 610391 to whom completed forms should be returned by 26th October, 1990 quoting Post No 0414.

An Equal Opportunities Employer. (4815)A

JOHN INNES CENTRE FOR PLANT SCIENCE RESEARCH

Applications are invited for a

PLANT BIOCHEMIST OR MOLECULAR BIOLOGIST

to join an EEC funded collaborative research programme on plant somatic embryogenesis. The successful applicant will work within the Cell Surface Group in the Department of Cell Biology and will be responsible for the molecular characterisation of a recently identified family of developmentally regulated cell surface proteoglycans. The work will link closely with other international groups in the programme. Further details can be obtained from Professor K R [illegible]; (tel: 0603 52571, fax: 0603 5[illegible]

This post, which will be available in November/December this year, will continue until the funding body withdraws its financial support. Appointment will be at the Higher Scientific Officer grade on the salary scale £11,586 to £16,176, according to qualifications and experience. Non-contributory superannuation.

Candidates should have a first or upper second class honours degree with a minimum of two years' experience preferably in either glycoprotein biochemistry or in the cloning and characterisation of plant genes.

Applications should be sent, together with a full CV and the names of two referees, to the Personnel Officer, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, quoting reference no CB/513 by 31st October, 1990. Equal Opportunities Employer. (4820)A

Chair

Department of Biochemistry and Molecular Biology

The Medical College of Georgia invites nominations and applications for the position of Professor and Chair of a newly restructured Department of Biochemistry and Molecular Biology in the School of Medicine. We seek a leader who can further develop and maintain academic excellence for this department. The successful candidate must have:

- an established record of major research accomplishment in the application of modern approaches to any area of biochemistry, molecular biology or cell biology relevant to human health and disease;
- the ability to attract and aid the development of first-rate faculty members; and
- a commitment to excellent medical and graduate education.

Applicants should provide a curriculum vitae, summary of professional goals, and the names of three references by Nov. 30, 1990 to:

R. W. Caldwell, Ph.D.
Biochemistry and Molecular Biology Search
Department of Pharmacology and Toxicology
Medical College of Georgia
Augusta, Georgia 30912.

Provisions of the Georgia Open-Records Act apply. (NW5921)A

THE MEDICAL COLLEGE OF GEORGIA
The Health Sciences University of the State of Georgia

An Affirmative Action/Equal Opportunity Employer

MEDICAL ONCOLOGIST/ HEMATOLOTIST

Outstanding opportunity for a well-trained clinical investigator to join the NIH, ACS-supported Human Immunology & Cancer Program in the Department of Medicine, University of Tennessee Medical Center at Knoxville (UTMC/K). Major responsibilities include active participation in patient care with research emphasis on monoclonal Ig-associated human diseases (multiple myeloma, lymphoma and related diseases) and especially amyloidosis.

The successful candidate will work in a well-equipped, state-of-the-art basic and clinical research facilities including hybridoma/Mab, flow cytometry, protein and peptide chemistry, immuno-chemistry, molecular biology, and computer laboratories. Excellent patient care services include a special dysproteinemia clinic, clinical research nurse support and advanced imaging capabilities. Position requires a minimum of 3 to 5 years of postdoctoral clinical and research experience and ability to conduct innovative research involving molecular/cellular immunology. Tenure-track position available immediately. Excellent compensation (including fringe benefits) and academic rank commensurate with education and experience. The Knoxville area offer the scientific resources of the University of Tennessee and the Oak Ridge National Laboratory, recreational opportunities provided by the surrounding Great Smoky and Cumberland Mountains and extensive TVA lake system; and cultural amenities including a symphony and chamber orchestra, a major fine arts museum, opera and theatre.

Letter of application, CV, and names and addresses of 3 reference sources should be sent to: Alan Solomon, MD, Professor of Medicine and Head, Human Immunology & Cancer Program, University of Tennessee Medical Center/Knoxville, 1924 Alcoa Highway, Knoxville, TN 37920. *The University of Tennessee is an affirmative action/Title IX /Section 504 employer.* (NW5924)A

NATIONAL VETERINARY INSTITUTE
NORWAY

The National Veterinary Institute is the central veterinary diagnostic laboratory in Norway, and serves as an advisory body for the agriculture authorities with regard to livestock disease control, and the administration of official regulations concerning the health of livestock, fish and game.

SENIOR RESEARCHER, SCIENTIST — Gene Mapping

At the joint department of Animal genetics at the Norwegian College of Veterinary Medicine/the National Veterinary Institute there is a vacant position as senior researcher, scientist.

The person to be employed will play a central role in our program on gene mapping in livestock species.

Scientific experience within genetics/molecular genetics is highly wanted. Research experience within the field of immunogenetics will also be considered relevant. Leadership qualifications and personal qualities will be emphasized as well. The staff of the department numbers about 25, and a wide range of objectives within the field of genetics is dealt with according to held responsibilities for teaching, research and genetic diagnostic service.

The position is financed through the Norwegian Agriculture Research Council's biotechnology program for a period of 3 years with possibilities for prolongation. The monthly salary amounts to NOK 21.700.-. (approximately USD: 3.550).

For further information please contact: Professor Oystein Lie, tel: 47 2 964782 or 47 2 964500. Fax 47 2 460034. Applications should be addressed to: The National Veterinary Institute, PO Box 8156 Dep, 0033 Oslo 1, Norway. Closing date for applications: 25th October, 1990. (W7818)A

FACULTY POSITION

The Laboratory of Genetics, University of Wisconsin, is expanding its research and teaching programs by recruitment of an Associate or Assistant Professor in mammalian or human genetics. All areas of mammalian genetics/molecular genetics will be considered, but applicants with research programs applicable to human disease are particularly welcome.

Send a curriculum vitae, a statement of research interests, three letters of recommendation, and representative reprints or preprints to Mammalian Genetics Search Committee, c/o Carter Denniston, 118 Genetics, University of Wisconsin, 445 Henry Mall, Madison, WI 53706. Application deadline is 15th November, 1990. *University of Wisconsin is an equal opportunity/affirmative action employer.* (NW5925)A

RESEARCH CENTRE FOR CANCER AND TRANSPLANTATION

Department of Pathology

UNIVERSITY OF MELBOURNE
Parkville, Victoria, Australia

RESEARCH FELLOWS GRADE 1 or 2

Several post-doctoral positions are available in the Research Centre for Cancer and Transplantation to work on various aspects of cell surface molecules. In particular molecular biological studies of Fc receptors and CD46. The Research Centre for Cancer and Transplantation is an active group with expertise in monoclonal antibodies, genetic engineering and immunoconjugates. The applicants should have experience in these or related areas and possess a PhD.

Salary is in the range Research Fellow Grade 1 ($28,792-$32,762) and Research Fellow Grade 2 ($33,163-$43,096) depending upon qualifications and experience.

It is envisaged the positions will be for up to 2 years in the first instance.

The Research Centre for Cancer and Transplantation will be relocating to establish a new research institute in Melbourne in the grounds of the Austin Hospital in Heidelberg in mid-1991.

Written applications, in duplicate, quoting position number, a telephone number for contact and the names, addresses and telephone numbers of two referees should be sent to: **Professor Ian F C McKenzie, MD, PhD, Director, Research Centre for Cancer and Transplantation, Department of Pathology, University of Melbourne, Parkville, Vic 3052, Australia. Telephone: 61 3 344 5839, Facsimile: 61 3 3475242.**

Closing date: 23rd November, 1990. (W7822)A

UNIVERSITY OF COLORADO

ATMOSPHERIC CHEMIST

The Department of Chemistry and Biochemistry and the Cooperative Institute for Research in Environmental Sciences (CIRES) seek to fill a position in the area of atmospheric chemistry. The tenure-track faculty position may start any time between January and September 1991. It is preferred to fill the position at the Assistant Professor level, but candidates will be considered at all levels. The incumbent will teach atmospheric, analytical or environmental chemistry courses and will be expected to develop a vigorous research program in atmospheric chemistry. We are particularly interested in candidates having field studies as a component of their research program. A PhD is required. Minimum 9-month salary is expected to be $36,000 for the position filled at the Assistant Professor level.

Send resume, transcripts and ask that 3 letters of reference be sent to Prof R E Sievers, Director of CIRES, Campus Box 216, University of Colorado, Boulder, CO 80309. First consideration will be given to applications for the position that are received by 1st November, 1990. The search will continue until the position is filled.

The University of Colorado, Boulder has a strong institutional commitment to the principle of diversity in all areas. In that spirit, we are particularly interested in receiving applications from a broad spectrum of people, including women, members of ethnic minorities and disabled individuals. *(NW5916)A*

The Department of Chemistry in the Faculty of Arts and Science at New York University

invites applications for a

TENURE-TRACK POSITION

in organic chemistry. A full-time faculty member with accomplished research and teaching interests in organic chemistry is sought. All applicants should submit a curriculum vitae, statement of research interests, and three letters of reference by 30th November, 1990, to: **Dr Neville Kallenbach, Department Chairman, Department of Chemistry, New York University, 4 Washington Place, New York, New York 10003**. New York University is an equal opportunity, affirmative action employer. Applications from minority candidates are strongly encouraged. (NW5917)A

FACULTY POSITION

MOLECULAR BIOLOGY/ IMMUNOLOGY

MEAKINS-CHRISTIE LABORATORIES
FACULTY OF MEDICINE
MCGILL UNIVERSITY

The Meakins-Christie Laboratories invites applications for a tenure-track position as Assistant or Associate Professor in Medicine. Candidates must have a PhD and/or MD with post-doctoral experience in molecular biology/immunology and are expected to develop an independent research program in the areas of airway disease, cystic fibrosis, lung injury or muscle function. The position will be opened in 1991 at salary commensurate with experience. In accordance with Canadian Immigration requirements, this advertisement is directed to Canadian citizens and permanent residents of Canada.

Applications, including CV, representative reprints, brief description of future research plans and names of three referees should be sent to Dr S Meisler, Search Committee, Chairman, MCL, 3626 St Urbain Street, Montreal, Quebec, H2X 2P2, Canada.

(NW5908)A

UNIVERSITY OF NEWCASTLE UPON TYNE

Department of Biochemistry and Genetics

RESEARCH ASSOCIATE

Applications are invited for a postdoctoral research appointment funded by the Medical Research Council to purify and study multi-drug-resistance proteins from normal (non-tumorous) bovine plasma membranes.

Salary will be up to £13,495 per annum on the Grade IA scale according to qualifications and experience. The appointment is for one year in the first instance.

Applications with full curriculum vitae and the names and addresses of three referees, should be sent as soon as possible to Dr I C West, Department of Biochemistry and Genetics, Catherine Cookson Building, Medical School, Newcastle upon Tyne NE2 4HH, from whom further information may be obtained. Tel: (091) 222 6537. (4827)A

DIRECTOR

INSTITUTE OF ANIMAL BEHAVIOR

Rutgers, The State University of New Jersey, Newark

The IAB is searching for a Director. Nominations or applications from individuals with an earned doctorate and distinguished achievement in psychobiology, justifying appointment as tenured professor, are invited.

Applications accepted until 31st December, 1990 with a curriculum vitae, description of current research activities, and three letters to: **Chair, Search Committee, Rutgers, The State University Graduate School — Dean's Office, 360 King Blvd, Newark NJ 07102.**

Rutgers is an Affirmative Action/Equal Opportunity Employer.

(NW5912)A

CANCER CELL BIOLOGY

The Department of Surgery, Cornell University Medical College invites applications for the position of **Research Associate** in a laboratory studying site specific metastasis. Ongoing projects in the laboratory seek to understand why the majority of solid tumors metastasize to lymph nodes with such facility. Experience in cell adhesion, immunologic and molecular biologic techniques would be advantageous, but not essential. The position is available immediately. Salary range $25,000-28,000 per annum. Informal inquiries should be directed to Dr Giles Whalen, Dept of Surgery (212-746-5434).

Applicants should send a curriculum vitae with the names of at least two references to: Veronica Johannesson, Dept of Surgery, 525 E 68th St, Rm F-739, New York, NY 10021.

(NW5909)A

HARVARD MEDICAL SCHO

EXPRESSION CLONING AND IMMUNOCYTOCHEMISTRY. Positions are available for enthusiastic **POSTDOCTORAL FELLOWS** to join a group actively involved in the molecular genetics of epithelial transport of glucose, amino acids and urea. Studies involve **Xenopus** oocyte expression, recombinant DNA techniques, protein chemistry and immunocytochemistry. Experience required in at least one of these fields. Send curriculum vitae and names of three references to: **Natthias Hediger, Ph.D., Renal Division, Brigham and Women's Hospital, 75 Francis Street, Boston MA 02115.** (NW5923)A

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Research Opportunities in Japan

NEDO

Introduction
Since October 1988, the Industrial Technology Department of the New Energy and Industrial Technology Development Organization (NEDO, an implementing agency of the Japanese Government) has been engaged in the implementation of a research facility development program. The purpose of the program is to estabish "Research Facility Development Corporations" for the promotion of research and development of advanced industrial technology. In particular, these corporations are devoted to the development of research facilities that are too expensive to be tackled by the private sector alone. The facilities thus developed are made available for the use of both domestic and foreign researchers.

Each Research Facility Development Corporation, which operates the facilities it develops, is funded by NEDO, private companies and local governments. NEDO promotes research and development projects that will make the maximum use of these corporations. At the same time, NEDO supports these corporations so that they will contribute to the progress of the international community through the development of advanced research facilities and industrial technology. Five Research Facility Development Corporations have been established, of which (1)-(3) are being introduced worldwide at this time [Nature Classified: October 4th, 11th and 18th].

Research Facility Development Corporations
(1) **Ion Engineering Center Corporation** (Hirakata City, Osaka Prefecture)
(2) **Research Center for the Industrial Utilization of Marine Organisms** (Kamaishi City, Iwate Prefecture, and Shimizu City, Shizuoka)
(3) **Japan Microgravity Center** (Kamisunagawa-cho, Hokkaido Prefecture)
(4) **Japan Ultrahigh-Temperature Materials Research Center** (Ube City, Yamaguchi Prefecture, and Tajimi City, Gifu Prefecture)
(5) **Applied Laser Engineering Center** (Nagaoka City, Niigata Prefecture)

Research Center for the Industrial Utilization of Marine Organisms

SPECIAL FEATURES

*** Unique Full-Equipped Research Facilities**
At present biotechnological research is advancing by leaps and bounds, however the bulk of such research thus far has been directed at terrestrial organisms.

The opening and utilization of these new research facilities make possible studies into areas of marine biology which were hitherto all but inaccessible to scholars.

*** Two Centers Offering Cold Current and Warm Current Research Capabilities**
The Japanese Archipelago is washed by both cold and warm currents which harbor distinct ecosystems and life forms.

The construction of two centers, one in Kamaishi City in Iwate Prefecture and the other in Shimizu City in Shizuoka Prefecture will facilitate study of the local marine life in two different marine ecosystems.

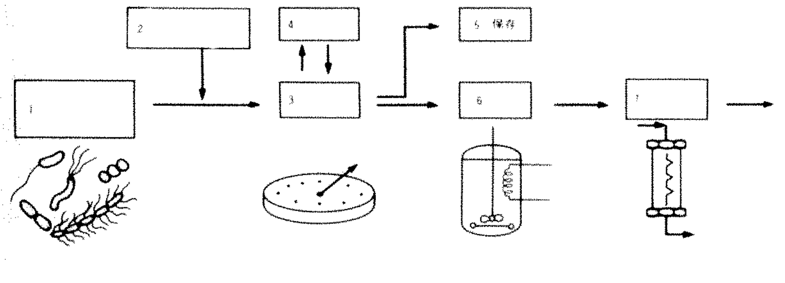
*** The Most Advanced Research Equipment Available**
The centers boast some of the most advanced research equipment available for a wide range of applications in biotechnology. Facilities include a bioengineering laboratory capable of handling radio isotopes, an aquatron which can simulate natural undersea environments and a wealth of high grade analytical equipment.

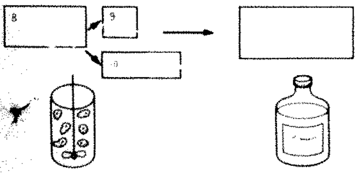
*** Pioneering in the New Field of Fine Chemicals**
The results of biotechnological research on marine organisms will facilitate the search for new utilizable substances and functions, and lead to improved technologies for cell culturing and breeding. The application of such advances is expected to greatly expand the field of fine chemicals.

THE KAMAISHI CENTER

The Kamaishi Center is slated to handle research relating to the exploration and elucidation of utilizable biological functions derived mainly from marine organisms cultured and bred in fresh sea water from the area. For culturing purposes, sterile culture systems of marine organisms (Aquatron), electron microscope, all kinds of aquariums and shaking bacterial incubator are equipped. Other bioresearch equipment include radio isotope and genetic engineering equipment, as well.

The area around the Kamaishi Center faces on Kamaishi Bay which is washed by a cold current. The surrounding area also plays host to the Otsuchi Marine Research Center of the Ocean Research Institute, University of Tokyo as well as the School of Fisheries Sciences of Kitasato University. As such, the center has real potential for becoming a focal point of marine research.





Steps involved in marine biotechnological research

COLLECTION OF MARINE MICROORGANISMS AND MICROALGAE
SEARCHING FOR USEFUL BIOLOGICAL SUBSTANCES (SCREENING)
PURIFICATION/SEPARATION
BREEDING/IMPROVING
PRESERVATION
CULTURING
SEPARATION/PURIFICATION
ESTABLISHMENT OF MANUFACTURING TECHNOLOGY
SYNTHESIS
MASS CULTURING
PRODUCTS

THE SHIMIZU CENTER

The Shimizu Center is slated to handle research into potentially utilizable substances found in marine organisms and the required separation and purification technology of such substances for application purposes. To satisfy these goals the Center is equipped with various devices for structural analysis such as a high performance nuclear magnetic resonator, an X-ray diffractometer and mass spectrometer. Other equipment include various biotechnological devices, genetic engineering equipment and equipment for the separation and purification of biological substances.

The Shimizu Center which based on Shimizu Bay is washed by a warm ocean current. At scenic Mihonomatsubara on the same seaside are to be found important academic institutions like The School of Marine Science and Technology of Tokai University, The National Research Institute of Far Seas Fisheries. As such this area has real potential for becoming an important center for marine studies.

* Enquiries should be made to the following address in Japan.
Research Center for the Industrial Utilization of Marine Organisms, 2-35-10 Hongo, Bunkyo-Ku, Tokyo 113, Japan. Tel: 03-5684-6210
Fax: 03-5684-6220

(W7790)A

MAMMALIAN CELL MOLECULAR GENETICIST

The newly reorganized **Department of Microbiology and Molecular Genetics** and **The Markey Center for Molecular Genetics** at the University of Vermont is continuing to expand its research base in molecular biology. A tenure track position at the assistant professor level is available in the area of molecular genetics related to mammalian cells and viruses. Areas of interest include, but are not limited to, (1) molecular virology, (2) fundamental mechanisms of DNA replication, recombination, gene expression, or protein synthesis, and (3) mechanisms of cell growth and differentiation. The candidate should be trained in genetics and molecular biology, and utilize modern approaches to problems of eukaryotic cell biology at the molecular level. A Ph.D. and postdoctoral experience are required. The expanded Department will occupy four floors in a new Microbiology Centre. Start-up funds and interim renovated space are available. Faculty members hold appointments in both the College of Agriculture and Life Sciences and the College of Medicine. The primary emphasis is on research; teaching responsibilities are to undergraduate, graduate and medical students. Research interests center on basic problems in molecular genetics with several groups focusing on cell and molecular biology of mammalian cells.

Send nominations and applications with curriculum vitae, present and future research plans and at least three letters of reference to:

Search Committee
Department of Microbiology and Molecular Genetics
Given Building, University of Vermont
Burlington, VT 05405

Review of applications will begin immediately and continue until the position is filled. Women and minorities are encouraged to apply. The University of Vermont is an Equal Opportunity/Affirmative Action Employer.

(NW5926)A

ASSISTANT PROFESSOR
Department of Microbiology
THE UNIVERSITY OF TENNESSEE
Knoxville, Tennessee

Qualifications: Doctoral degree and at least 2 years of postdoctoral experience in molecular virology or closely allied biological science, demonstrated research productivity in virology, and potential excellence in teaching.

Responsibilities: Graduate and undergraduate teaching in virology/molecular biology, establishment of a productive independent research program, and academic responsibilities normally associated with a tenure-track faculty position.

Starting date: 1st August, 1990.

Applicants should submit a letter of interest accompanied by curriculum vitae, a description of the applicant's research program and career objectives, and the names, addresses, and telephone numbers of three individuals who can evaluate the applicant's qualifications for the position. Applications from women and minorities are especially invited.

All material should be sent to: Dr Barry Rouse, Chairman, Search Committee, Department of Microbiology, University of Tennessee, Knoxville, TN 37996-0845. Nominations should be made as soon as possible; evaluation of applications will begin in December 1990.

The University of Tennessee is an Equal Opportunity/Affirmative Action/Title IX/ Section 504 Employer.

(NW5902)A

UNIVERSITY OF NEWCASTLE UPON TYNE
Department of Immunology
POSTDOCTORAL RESEARCH ASSOCIATE/JUNIOR RESEARCH ASSOCIATE

Applications are invited for 3 year appointment funded by the North of England Cancer Research Campaign, studying oncofetal antigen specific immunoregulation. Experience in celLular immunology including lymphocyte culture and cloning of the production of monoclonal antibodies would be advantageous. A postdoctoral appointment would be preferred but holders of good first degrees may be considered.

Starting salary will be up to £14,038 on the Research Associate (IA) scale (£11,399 pa -£18,165 pa) or on the Junior Research Associate (IB) scale (£11,399 pa-£13,495 pa) for a postgraduate appointee.

Applications, together with curriculum vitae and names and addresses of three referees, should be received not later than 26th October, 1990, by Dr R F Searle, Department of Immunology, The Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH from whom further particulars may be obtained. Informal enquirers may telephone 091 227 6625.

(4828)A

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE
SENIOR VIROLOGIST

Applications are invited for a tenured position at the Centre National de la Recherche Scientifique, to develop a research group involved in studies of neurotropic viruses or negative strand RNA viruses. The new group will be housed in the campus of Gif, a developed research space beautifully located in the south suburbs of Paris. The person appointed will be able to carry out its research in collaboration with another virology group, in a strongly interactive department.

Applicants should hold a doctoral degree and have a strong record of original research. Some working knowledge of French is necessary. Appointees are likely to be between 35-50. The salary will be according to the rule of the CNRS at the level of "Directeur de Recherche 2ème classe".

Further details may be obtained from Dr A Flamand, Laboratoire de Génétique des Virus, CNRS, 91198 Gif Sur Yvette Cedex, France (Tel 33 1 69 82 38 42 or Fax 33 1 69 07 04 21).

(W7812)A

CENTRAL BIRMINGHAM HEALTH AUTHORITY
QUEEN ELIZABETH HOSPITAL
DEPARTMENT OF MEDICINE
THE AMERSHAM RESEARCH BIOCHEMIST

The post, which is funded by Amersham International PLC, is a one year fixed contract which may be renewable. You will join an established programme of molecular biology in endocrinology within the Department of Medicine. The post involves the study of molecular mechanisms of thyroid hormone action; techniques to be used include cell culture, plasmid preparation and mRNA hybridization assays. You should be a graduate in a biological subject. Previous experience in molecular biology is not essential.

Salary: £9,239-£12,158 per annum.

Informal enquiries to Professor M C Sheppard or Dr J A Franklyn on ext 3361.

Application forms and job description from the Personnel Department, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH. Tel: 021 472 1311 ext 3003. Job Ref: 603.

Closing date: 31st October, 1990.

The Authority is Committed to Equal Opportunities in Employment.

(4807)A

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PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH
DIVISION OF PATHOLOGY
POST GRADUATE OR POST DOCTORAL RESEARCHER

A vacancy exists in the protein chemistry section for a post graduate or post doctora researcher to develop technologies for the purification of immunodeficiency virus antigen. The objectives of the project are to produce authentic virus proteins for research purposes and for the development of experimental vaccines. The post is full time, available immediately, initially for two years and funded by the MRC AIDS Directed Programme.

Candidates should have experience in protein purification techniques. Expertise in the area o[illegible]munoaffinity purification [illegible] FPLC would be desirable but [illegible] essential.

Further details and a job descr[illegible]tion can be obtained from Dr G [illegible] Farrar on ext 2560 and 2228.

The appointment will be made to either the Basic or Senior Scientist scales: £9,239-£12,158 or £12,644-£16,639 per annum respectively and will depend on age, qualifications and experience. The salary is currently under review as part of regarding to a new scientific scale.

Whitley Council terms and conditions apply.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG (Tel: 0980-610391, ext 2240), to whom completed forms should be returned by 26th October, 1990, quoting Post No 0322.

An Equal Opportunities Employer.

(4813)A

DEPARTMENT OF MEDICINE
UNIVERSITY OF CAMBRIDGE
RESEARCH TECHNICIANS

Applications are invited for two graduate research technicians to join a group investigating the molecular basis of human cytomegalovirus persistence and pathogenesis.

One, funded by the Wellcome Trust, will involve work on PCR of viral genes and analysis of the regulation of viral gene expression by DNA transfection and protein: DNA binding studies. Experience in molecular biological techniques would be an advantage.

The other post, funded by an MRC programme grant, will involve work on the T cell response to human cytomegalovirus – experience in cellular immunology is desirable but not essential.

Each post is tenable for 3 years in the first instance. Starting salary up to £9,135.

Applications, including CV and the names and addresses of two referees, should be sent to Mr John Holdon, Laboratory Manager, Department of Medicine, Level 5, Addenbrooke's Hospital Hills Road, Cambridge CB2 2QQ. Informal enquiries to Dr John Sinclair, Department of Medicine (0223) 336850. (4826)A

UNIVERSITY OF CAMBRIDGE
Department of Applied Mathematics and Theoretical Physics

Silver Street, Cambridge, England CB3 9EW
Telephone: Cambridge (0223) 337900
Telex: 81240 CAMSPLG. Fax: (0223) 337918

POSTDOCTORAL RESEARCH ASSOCIATES IN FLUID MECHANICS

Applications are invited for two postdoctoral research associates to work on mixing processes in stratified fluids. One post is for an experimentalist to study molecular mixing processes. The second post is for a numerical modeller to investigate interacting, gravity-driven flows. Both positions are within an active research group working on geophysical and environmental fluid dynamics, and are funded for three years.

Salary is in the range £10,699-£15,444 depending on experience and qualifications.

Applications should include a CV and the names of two referees, and be sent to Dr P F Linden, DAMTP, Silver Street, Cambridge CB3 9EW (Tel: 0223 337845) from whom further information can be obtained. (4844)A

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF OXFORD

POSTGRADUATE RESEARCH ASSISTANT RA1B

BR/339

Applications are invited for the above post, which is funded for three years by the Agricultural and Food Research Council. The aim of the project is to build a plant minichromosome. A simple plant cell with a small genome size, a unicellular green alga, will be used as a source of telomeres and centromeres.

Experience in yeast genetics or recombinant DNA work and a background in biochemistry, genetics or a related discipline would be an advantage.

Salary on the scale £10,699-£15,444 pa.

Applications, including a full curriculum vitae, and the names and addresses of two referees, should be sent to:

The Administrator

An Equal Opportunity Employer (4829)A

DEPT. OF BIOCHEMISTRY, SOUTH PARKS ROAD, OXFORD, OX1 3RE.

UNIVERSITY OF READING
School of Animal and Microbial Sciences
Department of Microbiology

POSTDOCTORAL RESEARCH FELLOW
(Up to £18,165 pa) Ref R9043

RESEARCH OFFICER
(Up to £13,495 pa) Ref R9044

TECHNICIAN GRADE D
(Up to £11,589 pa) Ref T40

These posts, funded by AFRC's initiative on the Biology of Spongiform Encephalopathies, are available for 3 years from April 1991 to work on the influence of prion protein variation on susceptibility to scrapie, BSE and related diseases. This work involves collaboration with other centres in the UK. Candidates should possess a PhD for the Fellowship, a good honours degree for the Officership, or a degree or HNC for the Technician. Experience of molecular biological techniques is required.

Further particulars may be obtained from Professor J W Almond, Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ. Tel: 0734 318901. Applicants should provide a curriculum vitae and the names of 2 academic referees. Apply, quoting appropriate ref no, for application form (UK applicants only) to Personnel Officer, University of Reading, Whiteknights, PO Box 217, Reading RG6 2AH. Tel: 0734 318754. (4801)A

UNIVERSITY OF WARWICK
Department of Biological Sciences
POSTDOCTORAL RESEARCH ASSISTANT

Funded by the SERC Invertebrate Neurobiology Initiative. To work on the analysis of cell lineage in the Drosophila nervous system using the yeast FLP recombination system (see Golic & Lindguist, Cell 59:499). Interest or experience in molecular biology, genetics or animal development would be an advantage.

Salary at either postdoctoral (1A: Salary scale: £11,399-£18,165 pa) or research assistant (1B: Salary scale: £11,399-£13,495 pa) level and is available for up to three years. Further particulars available from Dr C J O'Kane on 0203 523523 ext 2812/2559.

Application forms from Miss Nicole Freeman (0203 523502, direct line), Department of Biological Sciences, University of Warwick, Coventry CV4 7AL quoting Ref: 10/A/90/14 (please mark clearly on the envelope). Closing date for applications 1st November, 1990.

An equal opportunities employer. (4812)A

ANALYST

An opportunity has arisen to join the Quality Assurance Department of SPP, a member of the Dalgety Group of Companies, which is based at our premises in Standon in Hertfordshire. SPP market a range of technical bakery ingredients to bakeries and food manufacturers. Part of the range includes branded bakery products.

Reporting to the Laboratory Supervisor, the job will involve the analytical study of sample of all the products manufactured at our factories. We are particularly looking for a flexible, numerate person with high standards of presentation, good communication skills and attention to detail. The ideal candidate will have completed, or be about to complete, their studies in a Chemistry biased HNC qualification.

Salary will be negotiable, according to qualifications and experience: a Group pension scheme and 24 days holiday per annum are offered.

Applications with detailed CV: (No Agencies please)

Mrs P Bale,
Personnel Administration Manager,
SPP Cambridge,
Head Office & Technical Centre,
Station Road,
Cambridge CB1 2JN.
Tel: Cambridge (0223) 460666. (4831)A

ENDOWED CHAIR IN PLANT MOLECULAR BIOLOGY

Iowa State University invites applications for the Chair in the Molecular Biology/Genetics of maize endowed by Pioneer Hi-Bred International. We seek a research scientist with an established basic research program in molecular biology, genetics, and/or biochemistry to pursue research in plant molecular biology. The holder of the endowed chair will be expected to provide leadership in research in the plant sciences programs, especially in maize.

Opportunities exist for interactions with an ISU plant science research group, including geneticists, biochemists, plant breeders, and molecular biologists. The individual will have an important role in hiring new faculty in plant molecular biology and genetics. The recipient of the chair will receive generous start-up funds and annual support for research from the endowment. The individual will be given excellent space in the new molecular biology building and will have access to a large collection of superior quality germ plasm and genetic stocks for research in maize and other plants.

Applications are to be sent to D J Graves, Chair of the Search Committee, Department of Zoology and Genetics, Iowa State University, Ames, IA 50011 and should include a curriculum vitae, description of current and future research activities, and three letters of reference. Application deadline is 15th January, 1991 or until a suitable candidate is found.

Iowa State, an affirmative action/equal opportunity employer, encourages applications from women and minorities.

(NW5920)A

FELLOWSHIPS

EMBL

EUROPEAN MOLECULAR BIOLOGY LABORATORY

PhD Programme in Molecular, Cellular, Structural and Computational Biology

The European Molecular Biology Laboratory will award predoctoral fellowships in 1991 for University graduates wishing to study for a PhD at EMBL Heidelberg, Federal Republic of Germany, or at the EMBL Outstations in Hamburg, FRG, and Grenoble, France. Areas of study include: Biocomputing (theory and analysis of protein structure and genomic sequence information, development of computer methods for molecular biology, protein engineering and design), Biological Structures (structural and functional analysis of membrane proteins and assemblies, nucleic acid-protein interactions, cytoskeletal protein, protein folding), Cell Biology (protein and membrane traffic, membrane biogenesis, post-translational modifications, functional aspects of the nucleo- and cytoskeleton), Differentiation (oncogenes and growth control, developmental and tissue-specific gene expression, cell cycle regulation, mouse embryogenesis and homoeodomain genes, haemopoietic cell differentiation). Gene Expression (regulation of gene expression at the transcriptional and post-transcriptional levels, protein engineering, structure and function of small nuclear RNAs) and Instrumentation (technical innovations relevant to molecular, cellular and structural biology). At the Outstations, biological structures and dynamics are studied using the specialized techniques of synchrotron radiation (Hamburg) and neutron diffraction (Grenoble).

To be eligible candidates must hold, or anticipate receiving in 1991, a first or upper second class honours degree, a masters degree, a diploma or equivalent in biological sciences, physical sciences or mathematics.

Candidates must be citizens of the member states of EMBL or EMBC namely: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Israel, Italy, Netherlands, Norway, Spain, Sweden, Switzerland or the United Kingdom.

The deadline for receipt of completed applications is 31st January, 1991. Successful candidates will be notified by mid March 1991. Those awarded predoctoral fellowships will be expected to begin working at EMBL in 1991. The normal duration of the fellowships will be 3½ years.

Application forms and a brochure giving further information about the research at EMBL and the groups participating in the Laboratory's PhD programme are available on request from: **Dean of Graduate Studies, EMBL, Postfach 10.2209, D -6900 Heidelberg, Federal Republic of Germany.**

(W7817)E

PORTSMOUTH POLYTECHNIC

School of Biological Sciences — Biophysics

TWO RESEARCH FELLOWSHIPS IN DNA-PROTEIN INTERACTIONS

Funded by the Wellcome Trust, these posts are tenable for two years and are available immediately. The projects form part of an on-going research programme on the molecular basis of protein-nucleic acid recognition.

One post (Ref 1) is to work with Dr K Firman on an investigation of DNA binding domains in type 1 restriction endonucleases. Experience in recombinant DNA techniques would be advantageous but not essential.

The second post (Ref 2) is with Dr G G Kneale to join a group using biophysical, biochemical and site-directed mutagenesis techniques to probe the mechanism of the interaction of the fd gene 5 protein with DNA. Candidates with relevant experience in any area of biochemistry/molecular biology are encouraged to apply.

The closing date will be 25th October, 1990.

Salary will be on the Research Fellow scale, which currently starts at £13,674 per annum (under review). Informal enquiries may be directed to Dr Firman (0705 842059) or Dr Kneale (0705 842055). Application forms are available from the Personnel Office, Nuffield Centre, St Michael's Road, Portsmouth PO1 2ED. Tel: 0705 843257. (4811)E

Portsmouth Polytechnic pursues a policy of equality of opportunity. Applications are particularly welcome from people with disabilities.

UNIVERSITY OF EDINBURGH

Department of Biochemistry

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for an SERC-funded postdoctoral fellowship to study intracellular membrane fusion in yeast using a combination of biochemical and molecular genetic approaches. Candidates must have, or be about to obtain, a PhD in a relevant subject and should have research experience in biochemistry or molecular biology: applications from experienced biochemists who wish to broaden their expertise will be particularly welcome. The appointment, which is for three years, is available from 1st January, 1991, with salary in the RA 1A scale (£11,399-£18,165), placement being according to age and experience.

Further particulars of the project may be obtained by telephoning Dr Ala Boyd (031) 667 1011 ext 2483.

Applications in the form of a full CV with names and addresses two referees should be sent to Dr Alan Boyd, Department of Bio chemistry, Hugh Robson Building, George Square, Edinburgh EH 9XD. Closing date for applications is 26th October, 1990. Pleas quote reference no 5825.

(4802)E

GIRTON COLLEGE, CAMBRIDGE

Glaxo Junior Research Fellowship in Molecular Biology

Girton College invites applications for a Junior Research Fellow to work in any area in the field of Molecular Biology.

The tenure of the Glaxo Junior Research Fellowship will be for three years from 1st October, 1991. The Fellowship has a stipend of £9,900 per annum for post-doctoral candidates, pensionable under USS if the applicant so wishes. Any fees payable to the University will be funded by the Fellowship fund. Residence is provided, if required, at a moderate charge; commons (ie meals) are free of charge.

Further particulars and application forms are available from the Bursar,
GIRTON COLLEGE
Cambridge CB3 0JG
(all applications should be sent by 24th October, 1990).

(4821)E

FELLOWSHIPS
continued

THE UNIVERSITY OF LEEDS DEPARTMENT OF EARTH SCIENCES

POSTDOCTORAL RESEARCH FELLOWSHIP IN SEISMIC ARRAY PROCESSING

Applications are invited for the above post of Research Fellow in Geophysics, sponsored by Amoco (UK).

The successful candidate should possess a strong background in mathematics, physics or theoretical geophysics, and a PhD in an appropriate subject. The appointment is available immediately for a fixed period of up to three years. The person appointed will work on a new theoretical basis for seismic array processing of 3-component data using an integrated analysis of both polarisation and apparent velocity to discriminate between interfering events. Candidates from fields other than geophysics with knowledge of processing techniques for arrays of instruments (for example radio astronomy, acoustics or radar) will be considered.

Salary on the IA grade for Research and Analogous Staff (£11,399-£18,165), according to qualifications and relevant experience.

Informal enquires may be made to Professor D Gubbins. Tel: 0532 335255.

Application forms and further particulars may be obtained from and completed applications forwarded to the Registrar, The University of Leeds, Leeds LS2 9JT (Tel: 0532 333969 — direct line), quoting reference no 49/51. Fax: (0532) 334123.

Closing date for applications: [illegible]th November, 1990.

The University of Leeds is an equal opportunities employer.

(4800)E

continued on page 21

UNIVERSITY OF STRATHCLYDE

Department of Physiology and Pharmacology

POSTDOCTORAL RESEARCH FELLOW

Applications are invited from suitably qualified candidates for a Postdoctoral Research Fellowship tenable until 30th September, 1992 in the first instance. Applicants should hold a PhD in biochemistry, pharmacology or related discipline. The position is for research into the role, regulation and possible dysfunction of quanine nucleotide regulatory building proteins (G proteins) in inflammatory cells. Salary in range £11,399-£13,495 per annum.

Applications (Ref R45/90) with full CV and the names and addresses of three referees should be sent to **Professor I W Rodger, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW** who will be pleased to supply further details. Closing date for applications: 30th October, 1990. (4819)E

POSTDOCTORAL FELLOWSHIP

The Atherosclerosis & Lipid Research Division of the Dept of Internal Medicine is seeking two qualified MDs and/or Phds to work in the area of molecular genetics and lipoprotein metabolism. This is a funded project with a competitive salary range based on experience and expertise.

Start date would be 1st July, 1991, or as soon thereafter as possible. These projects seek to ascertain molecular defects of patients and to characterize consequences for lipoprotein metabolism.

Send CV and outline of research interests to: **Dr Gustav Schonfeld, Washington University School of Medicine, Atherosclerosis & Lipid Research Division, 4566 Scott Ave, Box 8046, St Louis, MO 63110.** (NW5913)E

RAMSAY MEMORIAL FELLOWSHIP FOR CHEMICAL RESEARCH

The Trustees will consider in January 1991 applications for the award of one or more Ramsay Fellowships, which may be co-sponsored by British Industry, and one Ramsay Fellowship co-sponsored by the Royal Commission for the Exhibition of 1851. The Fellowships are normally tenable in the United Kingdom for two years, for candidates with experience of post-doctoral research. It is desirable that the successful applicants would not have done more than two years post-doctoral work and would be not more than 28 years of age on taking up the appointment.

The value of the Fellowships will normally be parallel with the lower part of the Lecturer scale for British universities, plus an expenses grant of up to £100 per annum: they may be superannuable.

The successful applicants will normally be required to take up the Fellowship on or about 1st October 1991.

Particulars from the Joint Honorary Secretaries, Ramsay Memorial Fellowships Trust, University College London, Gower Street, London WC1E 6BT. Completed application forms must be received by 1st December, 1990. Equal Opportunities Employer. (4806)E

STUDENTSHIPS

INSTITUTE OF CHILD HEALTH
(University of London)
Department of Growth and Development

PhD STUDENTSHIP
(Minimum stipend £8,000 per annum)

Applications are invited for a PhD studentship to investigate the structure and function of the growth hormone-binding protein and its modulation of growth hormone activity.

Candidates should have been ordinarily resident in the UK for a minimum period of three years and possess a First or Upper Second Class Honours Degree in an appropriate subject.

Applications in the form of a CV and the names of two academic referees should be submitted by the 2nd November, 1990, to Dr D Morrell, Department of Growth and Development, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, from whom further information may be obtained (Tel: 071-242 9789 ext 2129).

(4846)F

GRANTS, SCHOLARSHIPS & AWARDS

UNIVERSITY OF OXFORD
SCHOLARSHIP FOR MEDICAL TRAINING

A scholarship is available for a well-qualified graduate aiming for medical training. The award covers tuition fees and a generous contribution towards maintenance for the pre-clinical course only.

Full details are available from the Graduate Admissions Office, University Offices, Wellington Square, Oxford OX1 2JD. The latest date for receipt of applications is 31st October, 1990.

(4809)H

POSTDOCTORAL AWARDS
MARINE SCIENCE

The Australian Institute of Marine Science, located at Cape Ferguson (a coastal site 50 km from Townsville, Queensland, Australia and adjacent to the central section of the Great Barrier Reef), is offering Postdoctoral Awards, each of 2 years, for research by scientists in the early stages of their postdoctoral career, who are of exceptional promise and have shown proven capacity for original work in marine science research.

AIMS conducts research in four major research programs:

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Coral Reef Studies;
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CONDITIONS: A stipend of $A33,445 p.a. is payable plus assistance with relocation to Townsville. An information package detailing conditions of employment and the scientific activities of the Institute is available from the Personnel Manager +61.77.789 319. Fax No. +61.77.725 852.

APPLICATIONS: Candidates should submit a full curriculum vitae, a 2 - 3 page outline of a project investigation and support requirements. Three professional referee reports should also be arranged. Applications and referee reports must be received by the Secretary, Australian Institute of Marine Science, PMB No. 3, Townsville MC, Qld 4810 Australia no later than 4 January, 1991.

Candidates are encouraged to seek additional information on the Institute and its programs by telephoning the Director +61.77.789 211. Fax No. +61.77.789 386. (W7814)N

AIMS is an EEO employer.

COURSES/CONFERENCES/SEMINARS/ SYMPOSIA & WORKSHOPS

PROTEIN AND DNA COURSES

Organised by **Harish Patel & Alan D. B. Malcolm**
Department of Biochemistry, Charing Cross and
Westminster Medical School, Fulham Palace Road,
LONDON W6 8RF. Tel: 081 846 7048 Fax: 081 846 7066
In conjunction with **Pharmacia Ltd**

PROTEIN ELECTROPHORESIS COURSE
13th & 14th December, 1990

This course will consist of lectures and practicals. The various aspects of electrophoresis techniques and their applications will be discussed including:
1. Polyacrylamide Gel Electrophoresis, **2.** Isoelectric Focusing, **3.** Immunoelectrophoresis, **4.** Two-Dimensional Electrophoresis, **5.** Protein Blotting Techniques, **6.** Densitometry.
Places limited to 24 participants.

GENERAL MOLECULAR BIOLOGY TECHNIQUES
7th & 8th January, 1991

This course will consist of lectures and practicals. The various aspects of general Molecular Biology techniques will be discussed including:
1. Oligonucleotide Synthesis & Purification, **2.** DNA Sequencing & Troubleshooting, **3.** PCR, **4.** Nucleic Acid Blotting, **5.** Nucleic Acid Hybridisation.
Places limited to 24 participants.

Registration Fee:
£120 for non profit making organisations
£160 for profit making organisations
(10% discount on registering for both courses).
Morning Coffee, Lunch, Afternoon Tea included in fee.
For further details contact: Shirley Line at above address.

(4845)C

NATURE CONFERENCE

NEW HORIZONS IN IMMUNOLOGY

15-16 November 1990
Boston,
at the Copley Plaza Hotel

CONFERENCE SESSIONS

1. TOLERANCE AND THE IMMUNE REPERTOIRE
2. ANTIGEN PROCESSING AND PRESENTATION
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REGISTRATION FEES

Standard rate including reception: $350
Special rates for full-time academics and government employees only: $300

Contact: Diana Berger, Nature Publishing Company,
65 Bleeker Street, New York, NY 10012-2467 USA.
(Tel: 212-477-9600)

(NW5629)C

continued on page 23

THE EUROPEAN SCHOOL OF HAEMATOLOGY

1991 PROGRAM

STUDY SESSIONS
(courses conducted in English)

13th-17th May, 1991
AUTOLOGOUS STEM CELL TRANSPLANTATION IN HAEMATOLOGICAL MALIGNANCIES
Chairmen: P HERVE (FR) & B LOWENBERG (NL)
Venue: Chateau de Maffliers, France

24th-28th June, 1991
CYTOGENETICS AND GENETICS IN MALIGNANT BLOOD DISORDERS
Chairpersons: J TANZER (FR) & C MECUCCI (IT)
Venue: Chateau de Maffliers, France

18th-22nd November, 1991
ADHESIVE MOLECULES
Chairmen: G MARGUERIE (FR) & A FISCHER (FR)
Venue: Chateau de Maffliers, France

22nd-24th April, 1991
MYELODYSPLASTIC SYNDROMES
in collaboration with the Haematology Collaborating Group at the Karolinska Hospital (SAHKS) in Stockholm
Chairmen: P REIZENSTEIN (SE), A OST (SE)
C SULTAN (FR)
Venue: Stockholm, Sweden

THE EUROPEAN TUTORIAL ON HAEMATOPATHOLOGY
21st-24th October, 1991
Chairmen: D CATOVSKY (GB), D Y MASON (GB), G FLANDRIN (FR) & J DIEBOLD (FR)
This new ESH endeavor will have a theoretical and a practical aspect. Each participant will work with his own individual microscope provided by the ESH. This course will be conducted in English.

SEMINAIRES DE TECHNOLOGIE APPLIQUEE
(courses conducted in French)

21-24 Janvier 1991
AUTOMATION EN HEMATOLOGIE
Organisé par C SULTAN (FR)
Lieu: Créteil, France

15-18 Avril, 1991
EXPLORATION DU SYSTEME FIBRINOLYTIQUE
Organisé par I JUHAN-VAGUE
Lieu: Marseille, France

4-7 Novembre 1991
HEMATOLOGIE MOLECULAIRE
Organisé par F SIGAUX
Lieu: Paris, France

Les Séminaires de Technologie Appliquée ESH mettent l'accent sur les travaux pratiques en laboratoire; les exposés théoriques sont suivis par des manipulations pratiques.

FOR FURTHER INFORMATION PLEASE CONTACT THE EUROPEAN SCHOOL OF HAEMATOLOGY, CENTRE HAYEM, HOPITAL SAINT-LOUIS, PARIS CEDEX 10, FRANCE
TELEPHONE: (1) 42 06 32 60; FAX: (1) 42 41 14 70

(W7819)V

RESEARCH OPPORTUNITY IN JAPAN

MITSUBISHI PETROCHEMICAL CO LTD (MPC)

Tsukuba Research Center, Japan

the largest petrochemical company in Japan

is offering a two/three year

Postdoctoral Fellowship

We are seeking an outstanding investigator with an interest in experimental analysis of gene expression in bacteria.

The successful candidate will be a member of a highly motivated group dedicated to research and development in a number of exciting project areas. The principal task of the candidate will be to study control of gene expression in bacteria.

We offer excellent facilities and research opportunities together with an attractive stipend ($30,000 to $50,000 per year depending on experience) and benefits package. Interdisciplinary and collaborative research, and publication of experimental results, are strongly encouraged.

Interested candidates with experience in gene expression in bacteria should submit their CV, names of references, and a list of publications, as soon as possible, but by 30th November to:

Attn: Manager, Mr. Kenji Shimotsu
The Personnel Department
Mitsubishi Petrochemical Co Ltd
Tsukuba Research Centre
Amimachi-chuo 8-3-1, Inashiki-gun, 300-03
Japan
Tel 81-298-87-1011
Fax 81-298-87-3259

Chief Scientist: Dr. Hideaki Yukawa

(W7788)E

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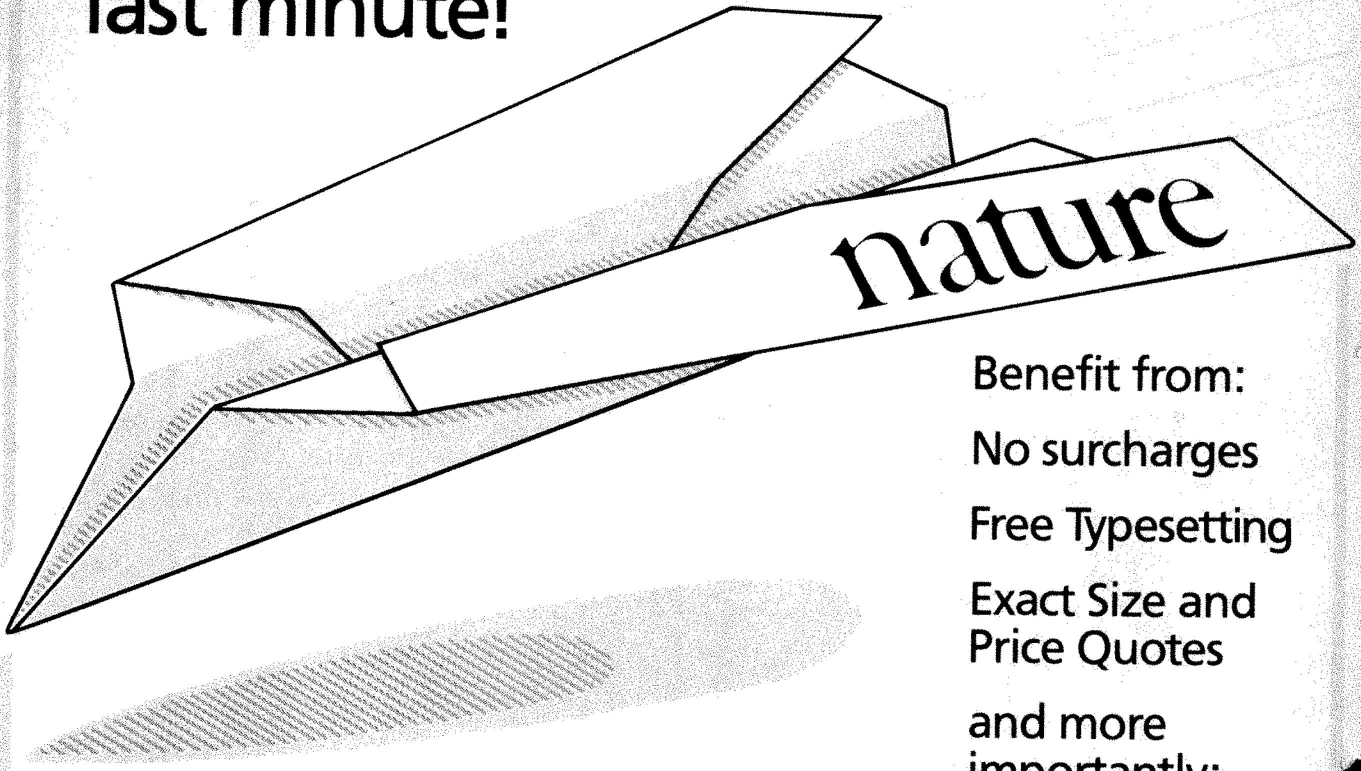

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International Symposium

BIOTECHNOLOGY OF GROWTH FACTORS:

Vascular and Nervous Systems

Milan (Italy), May 13-15, 1991

Preliminary list of topics and speakers:

General Introductory Session: Growth Factors Mechanisms
J.A. Massagué *(USA)*, L.T. Williams *(USA)*, N.K. Tonks *(USA)*

Angiogenesis
D.B. Rifkin *(USA)*, I. Vlodavsky *(Israel)*, H.M. Blau *(USA)*, B. Ensoli *(USA)*, E. Wagner *(Austria)*

Endothelial Cell Activation and Interaction
E. Dejana *(Italy)*, D. Haskard *(UK)*, U. Ryan *(USA)*

Differentiation and Function of the Nervous Central System
K. Unsicker *(FRG)*, P. Calissano *(Italy)*, G.D. Yancopoulos *(USA)*

Satellite Meeting

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Milan (Italy), May 13-14, 1991

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Biotechnology

(W7815)M

Symposium on:

THE ROLE OF CIRCULATING AND LOCAL FACTORS IN HYPERTENSION

LONDON, SATURDAY 16TH FEBRUARY 1991

School of Pharmacy, University of London,
29/39 Brunswick Square, London WC1

Invited lectures and free poster communications of new work on:

1. *Circulating vasodilators*
2. *Circulating vasoconstrictors*
3. *Local vascular regulation, including growth factors*

Abstract deadline: Friday 7th December, 1990

Titles	**Invited speakers**
Endogenous digitalis: the link between dietary sodium and high blood pressure	*M P Blaustein, Baltimore, USA*
Role of atrial natriuretic peptides: insight from studies of endopeptidase inhibitors	*E J Sybertz, New Jersey, USA*
Genetic manipulation of the renin-angiotensin system in transgenic animals	*J Mullins, Edinburgh, UK*
Pathogenic role of growth factors and vasoconstrictor peptides	*T Scott-Burden, Houston, USA*

Registration fee:
£10 – members of London Hypertension Society
£20 – non-members
Fee will be waived for presenting authors

Registration and abstract forms:
Sally Prestwich, London Hypertension Society, c/o Dept of Pharmacology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE.
Tel: 44 81 672 9944 ext 55657
Fax: 44 81 767 4696.

(4840)M

U.S. Postal Service

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Required by 39 U.S.C. 3685

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(If changed, publisher must submit explanation of change with this statement.)

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PS Form 3526, Feb. 1989 (See instructions on reverse)

(NW5899)J

120 YEARS AGO

I find that the "Address" which it was my duty to deliver at Liverpool fills thirteen columns of Nature. The "Reply" with which Dr. Bastian has favoured you occupies fifteen columns, and yet professes to deal with only the first portion of the "Address." Between us, therefore, I should imagine that both you and your readers must have had enough of the subject; and, so far as my own feeling is concerned, I should be disposed to leave both Dr. Bastian and his reply to the benign and Lethean influences of Time.

But I am credibly informed that there are persons upon whom Dr. Bastian's really wonderful effluence of words weighs as much as if it were charged with solid statements and accurate reasonings; and I am further told that it is my duty to the public to state why such distinguished special pleading makes not the least impression on my mind. With your permission, therefore, I will do so in the briefest possible manner.

The first half of Dr. Bastian's "Reply" occupies seven columns of your number for the 22nd of September. In all this wilderness of words there is but one paragraph which appears to me to be worth serious notice. It is this:—

"In the first place, he does not attempt to deny — he does not even allude to the fact — that *living things may and do arise as minutest visible specks, in solutions in which, but a few hours before, no such specks were to be seen.* And this is in itself a very remarkable omission. The statement must be true or false — and if true, as I and others affirm, the question which Professor Huxley has set himself to discuss is no longer one of such a simple nature as he represents it to be. It is henceforth settled that as far as *visible* germs are concerned, living beings can come into being without them."

If I did not allude to the assertion which Dr. Bastian has put in italics — it is because it bears absurdity written upon its face to any one who has seriously considered the conditions of microscopic observation. I have tried over and over again to obtain a drop of a solution which should be optically pure, or absolutely free from distinguishable solid particles, when viewed under a power of 1,200 diameters in the ordinary way. I have never succeeded; and, considering the conditions of observation, I never expect to succeed. And though I hesitate to speak with the air of confident authority which sits so well on Dr. Bastian, I venture to doubt whether he ever has prepared, or ever will prepare, a solution, in a drop of which no "minutest visible specks" are to be seen by a careful searcher.

"Dr. Bastian and Spontaneous Generation", T. H. Huxley.
From *Nature* October 13, 1870.

100 YEARS AGO

The phenomenon of globular lightning was imitated by M. Plante, it will be remembered, with his secondary batteries. It has been recently shown by Herr von Lepel (*Met. Zeits.*), that this can also be done with so-called statical electricity, obtained from an influence-machine. Two thin brass-wire points from the poles of a powerful machine being held at a certain distance from the opposite sides of an insulated plate of mica, ebonite, glass, or the like, there appear small red luminous balls, which move about, now quickly, now slowly, and are sometimes still. Even better effects were had with a glass or paper disk which had been sprayed with paraffin. Small particles of liquid or dust seem to be the carriers of the light.

Naturalists will read with interest a paper in *Humboldt* for September, in which Prof. Forel, of Zürich, gives the results of a visit he lately paid to Tunis and Eastern Algeria, chiefly to observe the ants there. Looking from a ship at the dreary grey wastes, and the large date-palm oasis of Gabes, one fancies all animal life must be concentrated under the palms. But reall
and hardly anything singular; while t
round each of the poor, small, spars
other insects, many of them with st
liarities. Some live on excrement of c
plants, and some prey on other anim
hill he found that several ants had a
the lower part of an antenna; in some
On examination, this fell off, and w
which evidently clings there as guest
probably licked by the ant. The host
about this little creature, which, b
accompany the ant in its wanderings
From *Nature* October 16, 1890.

50 YEARS

The October issue of the *British J*
instructive article by Brevet-Colone
G. S. Chopra of the School of Tropi
present position of hemp drug addi
India are at present used in three f
taken as a beverage, while *ganja*, whi
more potent, and *charas*, the effects o
those produced by *ganja*, are mostly
hemp plant which grows wild in Nor
slopes of the Himalayas is strictly co
The total consumption of hemp drug
35 amounted to 1,031,496 lb.

According to the writers there are
1,000,000 hemp drug addicts in Indi
cent of the total population.

It has been considered that during
excrete gonadotropic hormones in hi
and that pregnant Equidæ do not,
gonadotropic substances may be inc
expected that antelopes would give

We had occasion to examine the u
giraffes and our preliminary results t
of gonadotrophic hormones during
primates as is generally assumed, bu
"Gonadotropic Hormones in the
Wilkinson and P. de Fremery.
From *Nature* October 12, 1940.

10 YEARS

War is the greatest immediate threa
destruction of its fragile water suppl
At the end of this month local mar
nextdoor to the Iraq/Iran conflict, t
up to $5 million over two years to fin
to the waters of the Gulf.

The long-term development of th
recently recognized, depends on p
ment of the Gulf and its coastal re
ecology, oceanography or meteorol
needed for agriculture, coastal indu
highest density of tankers in any oce
and many industries such as desali
ciated chloralkali works (using m
smelters (emitting hydrogen fluori
ammonia), and refineries (sulphu
belch out completely uncontrolled
frequently tainted with oil, and city e
markedly in the past two or three ye
From *Nature* October 9, 1980.

IN NEXT WE

Living with toxic waste ■ Mole
■ A mid-ocean ridge in three
eclipsing millisecond pulsar ■
Barrier Reef ■ Novel informa
visual defects

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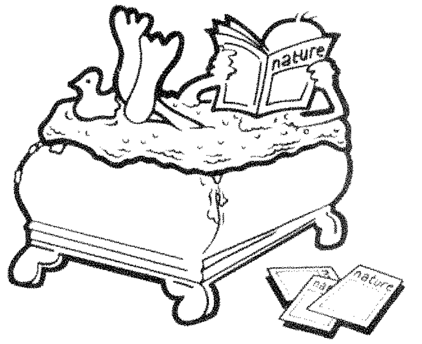

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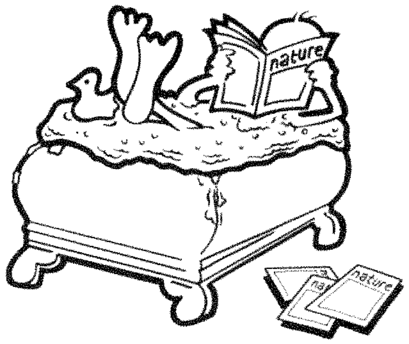

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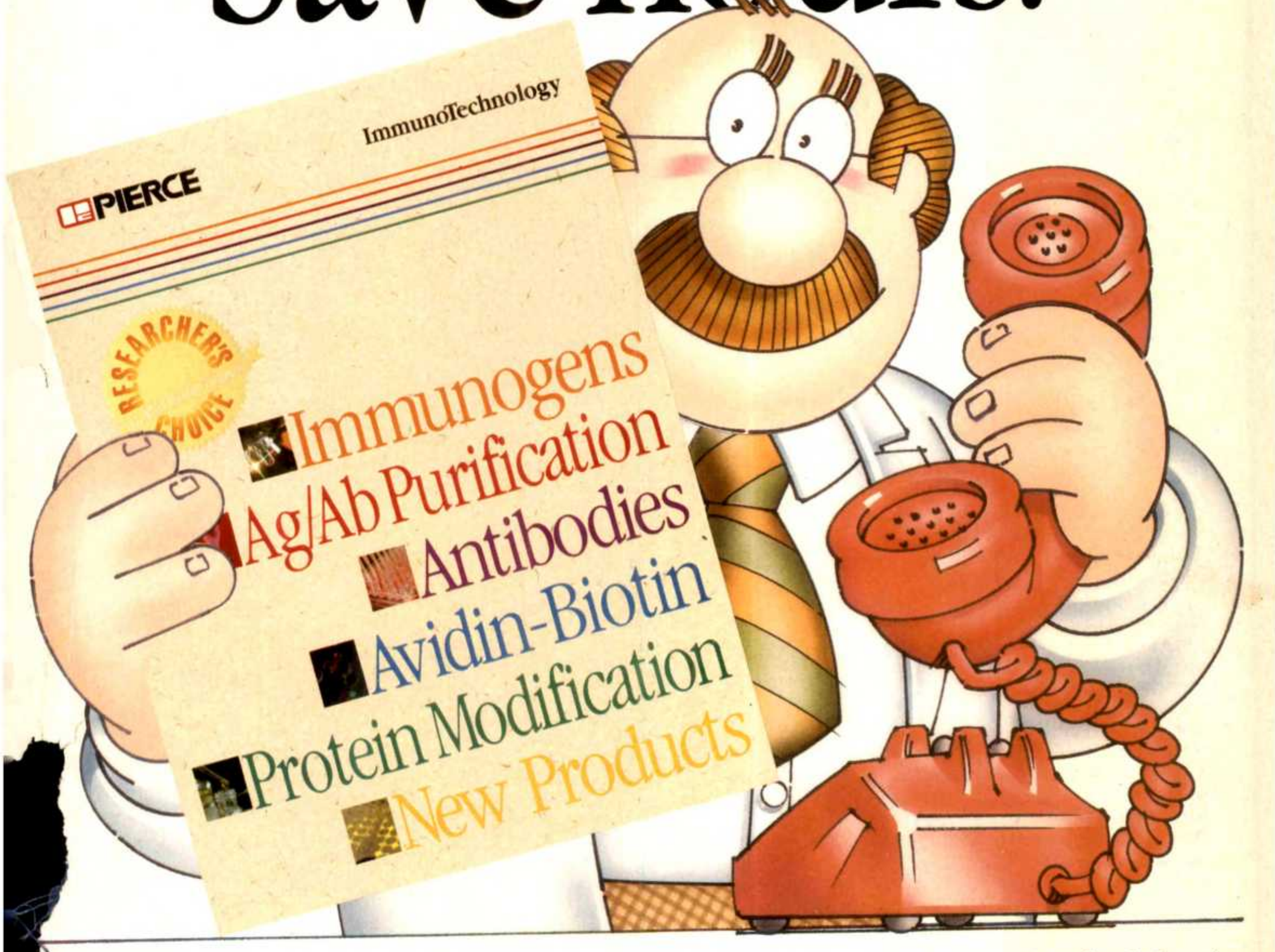

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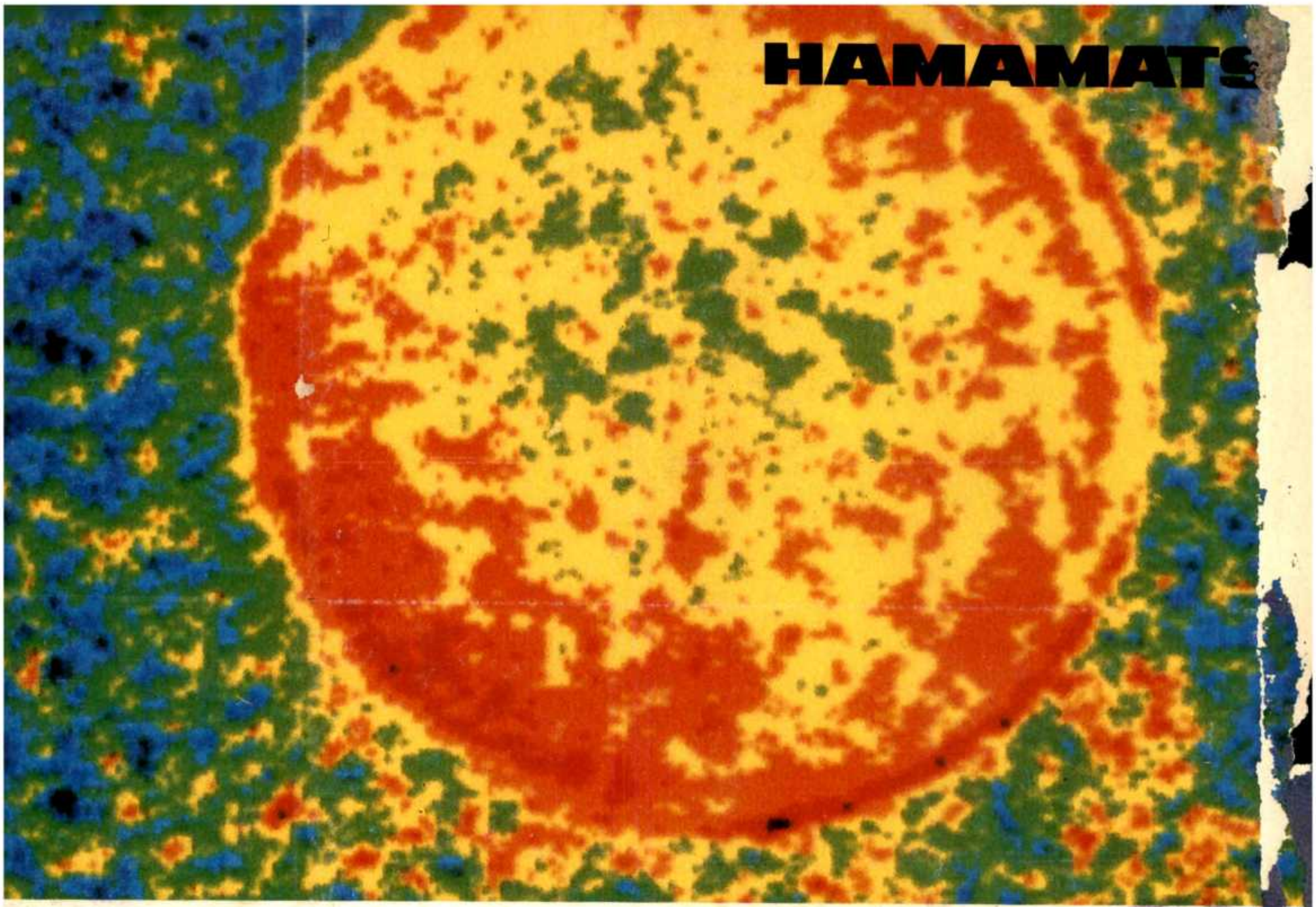

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